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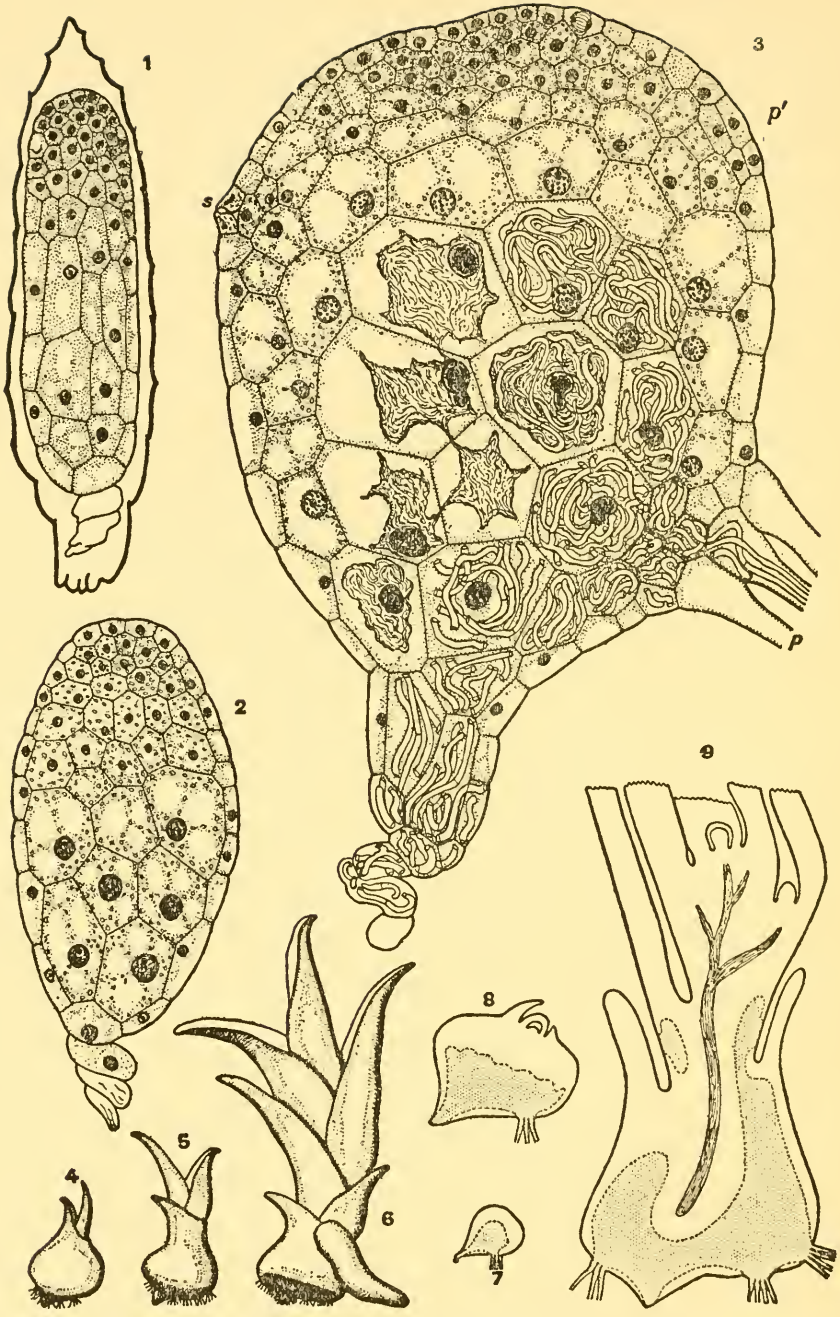
Arthur Pierson Kelley was born in the village of Malvern, in the hills suburban to Philadelphia, on August 15, 1897. He studied at the University of Pennsylvania, graduating with the degree of B.S. in Biol. in 1920. At the same University he acquired the degrees of M.A. (1921) and Ph.D. (1923). Later he studied plant physiology under Dr. Livingston at The Johns Hopkins University. Commencing as a teacher, he was successively instructor and assistant professor of botany at Rutgers University. Thereafter he joined the United States Forest Service hoping to find more time for research on mycorrhizae. In an effort to devote himself more fully to this work he later developed his private biological station and herbarium at Landenberg, Pennsylvania. From here he produced his digest of mycorrhizal literature which has become widely known. Dr. Kelley is now chiefly engaged in farm life and the reconstruction of historical farm houses, pursuits which, he writes, often leave too little time for the study of mycotrophy. Dr. Kelley's early publications dealt with soil acidity in relation to plant distribution, later papers with other ecological problems and the various aspects of mycotrophy.

• A NEW SERIES OF PLANT SCIENCE BOOKS •

edited by Frans Verdoorn

Volume XXII

MYCOTROPHY in PLANTS



FRONTISPIECE.—Development of an *Odontoglossum* as figured by NOEL BERNARD in 1909. Figure 1 is a sectional view of seed; the remaining figures are habit sketches of embryos and seedlings, the last two indicating regions of fungal infection by shading. Figure 3 is of special interest because it shows neatly coils of the fungus (considered *Rhizoctonia lanuginosa*), which has entered by an "absorbing hair". It shows also amoeboid condition of nucleus of infected cells. Figure 2 shows by vivid contrast the condition of an *uninfected* embryo at 4 months of age, whereas the embryo of figure 3 is but one month old.

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MYCOTROPHY *in* PLANTS

Lectures on the Biology of Mycorrhizae and related structures

by

ARTHUR P. KELLEY, PH.D.
Landenberg, Pennsylvania

1950

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PREFACE

Since this little volume was started more work has been done on mycotrophy. Yet the status of the subject remains unchanged. It is still considered by most botanists—who are the only people to whom even the word is familiar—as a specialty which should be listed under the general heading of pathology. Mostly it is not listed at all. Mycorrhizae are still considered abnormal structures occurring chiefly on pine roots; while by some curious aberration the mycotrophic structures found in prothallia and in rhizomes are likewise termed mycorrhizae. Fungus-roots are found in rootless plants! There is, moreover, a common impulse to lump all symbiotic phenomena together, as odds and ends and thrown together in a heap, so that the mycotrophist (if such a term may be used) is expected to be interested in cases of algae growing in higher plants, or of small insects found living in plant tissues. It is hoped that this book, in spite of its obvious faults, may serve to show how mycotrophy is separated from other phenomena, and how widespread is the mycotrophic habit.

Europe continues to be the center of mycotrophic study. In spite of war, a considerable number of papers has appeared in this part of the world in the last few years. Only a few papers on the subject have been published on the other continents.

Tree mycorrhizae continue to attract attention. Björkman (1944) has made important studies in Sweden. In Poland, Dominik (1946) has published interesting papers on fruit-tree mycorrhizae which were produced on all trees studied. Endotrophic mycorrhizae which were stimulated by applications of farmyard manure to the soil were found by Sabet (1946) on *Citrus* in Egypt, while Smith (1944/45) in Queensland found that "decline" in mandarin was associated with depletion of the soil and consequent interference with mycorrhizal activity. These results lend support to the idea that internal processes in at least certain trees can be controlled by appropriate manipulation of the habitat.

Forest tree mycorrhizae receive continued attention from two of the principal investigators of mycotrophy. RAYNER (1941) has summarized her own and related researches on the effect of composts on tree growth. MELIN's (1946) attention has been turned especially to growth and anti-bacterial substances with respect to mycotrophy. Recently fallen leaf-litter was found to contain water-soluble substances that promoted the growth of litter-decomposing and mycorrhizal fungi. Cognate studies have been made by HARLEY but his latest paper was not available at this writing.

Synthesis of *Boletus* with pine seedlings to form synthetic mycorrhizae was performed by FERREIRA (1941), thus confirming the discoveries of a number of earlier investigators. What is said to be the first synthesis of alder nodules was accomplished by PLOTHO (1944).

Obligate symbiosis is still questioned, but it is claimed by BOSE (1947) for *Casuarina* under the name of hereditary symbiosis. Mycelium extended into every organ of the tree, spreading from the seed-coats.

Among herbaceous plants, orchid mycotrophy claims the attention of BURGEFF (1943), and SPRAU (1939). The Burmannias and their mycotrophy were investigated by CIFERRI; and at his laboratory at Pavia there is active interest in our subject. To the several papers on mycotrophy in halophytes was added one by FRIES (1944): nine out of ten halophytes examined were mycotrophic. Again, in gametophytes of the fern *Botrichium,* NARYANA (1939) described endophytic infection. So, too, in hepatics described by PEYRONEL (1942) from Italy: double infestations occur and the symbiosis is much affected by edaphic conditions, but in general the relation seems to be of mutual benefit. A noteworthy contribution to the study of herbaceous mycotrophs was made by BARRETT (1947), who succeedeed in isolating *Rhizophagus* in pure culture.

There is a continually increasing emphasis on the physiology of mycotrophy, and several contributions to this subject have been made recently. From a yellow *Corticium* a pigment called "corticrocin" was isolated (ERDTMAN, 1947), "the first n-polyneoid diacid found in Nature." Another paper reverted to the Stahlian concept of stomata in relation to mycotrophy. It will be recalled that STAHL believed mycotrophs had a reduced number of stomata and a limited transpiration stream, mycotrophy supplying the nutrients otherwise supplied by photosynthesis. A recent investigation (LINSBAUER & ZIEGENSPECK, n.d.) concludes that amongst mycotrophs there is a significant reduction in number and formation of stomata. Extreme mycotrophs resemble holoparasites. A third physiological paper

(Prat, 1945) treats of gradients in mycotrophic plant tissues. Resistance to parasites is an important function in plants. In mycotrophy the gradient of resistance to the mycelium is slowly progressive. Mycelium penetrating toward the apex is always checked, for the axial gradient of resistance is directed toward the point of the root and is progressive; but the radial gradient of resistance is abruptly corrected at the level of the endodermis. Chemical barriers are more efficacious in developing resistance than physiological barriers. The gradients vary according to season. Still other papers (Magrou, 1944, 1946) deal with tuberisation and the factors which control it.

Acclimatisation has been proved aided by symbiosis. At Angers (Blaringhem, 1937), building a well-aerated humus layer favourable to growth of symbiotic fungi aided the acclimating of 400 spp. of conifers and 150 forms of oak, which proceeded to make 3 to 5 times the growth of the finest specimens of these species in their native haunts.

Two general papers on mycotrophic symbiosis have appeared recently. In one (Owen, 1947), symbiosis is examined with reference to the true character of the symbionts. It is concluded that true mutualism exists between nodule bacteria and legumes, and perhaps with mycorrhizae. But orchids are considered dependent on fungi, since the fungi can live apart saprophytically. With characteristic thoroughness Burgeff (1943) has analyzed mycotrophic phenomena and presented a new classification of them. Realizing that their most important function is material exchange which is dependent on the union of tissues of both components, he classifies "mycorrhizae" as: (1) *Tolypophagous,* in which rhizoctonial fungi form pelotons that are digested with release of fat, glycogen and nitrogenous material into the orchid plant that harbours them. (2) *Thamniscophagous,* in which arbuscles are digested, leaving sporangioles as excreta. Very widely distributed in green plants, including ferns and liverworts. In certain ferns and colorless saprophytes the arbuscles go over to the preceding form and are hence termed *thamniscotolypophagous*(!) The fungi appear to be Endogonaceae, hence the mycorrhizae are called phycomycetoid. (3) *Ptyophagous.* Found in such plants as *Gastrodia* and *Monotropa,* these "mycorrhizae" show resorption of materials released into cell by fungal hyphae. Free fungal bodies called "phytosomes" are formed which, being resorbed, leave "Exkretkörper". (4) *Chylophagous.* Sap resorption occurs in subterranean colorless and saprophytic prothallia of *Lycopodium.* There is no digestion and there are no excretion bodies. Sap is exuded by guttation of hyphae into intercellular spaces. (5) *Halmophagous.*

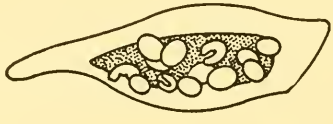
Included are the ectotrophic mycorrhizae which have mantle and Hartig net. There is said to be resorption of nutrient salts. These mycorrhizae are obligatory for many forest trees. With some species, *ectendotrophic* forms are found.

Selected chapters of the manuscript were read by Dr. G. R. Bisby, of the Imperial Mycological Institute; by Dr. D. T. MacDougal; by Dr. M. C. Rayner, of Bedford College; and by Dr. H. E. Young, Dept. of Agriculture and Stock, Queensland. None of these is responsible for views expressed by the author. Above all, the author is indebted to Dr. Frans Verdoorn, whose unflagging patience has brought the book through difficult times.

The Author

Dr. J. A. Hijner of the Netherlands, who worked at the California Institute of Technology, during 1948, just sent us a preliminary report in which he claims, on the basis of experimental data, that vitamins are essential for growth of *Rhizoctonia* isolated from *Cymbidium;* specifically, folic acid or para-aminobenzoic acid with thiamin. He considers that in nature these growth substances are supplied by germinating orchid seeds, which are known to contain them. He also points out localization of fungi in tissues of the host which he believes due to antibiotics produced by the host.

Attention might still be drawn to the small symposium edited by L. Blaringhem at the occasion of the Exposition Internationale, Paris (1937) :—Symbiose et Parasitisme, l'oeuvre de Noël Bernard. 89 pp. Paris: Masson. Other very recent publications which have not been referred to in the book:—Bahme, R. B. (1949) : Nicotinic acid as a growth factor for certain orchid embryos. Science 109:522-3. — Baumgärtel, T. (1940) : Mikrobielle Symbiosen im Pflanzen- und Tierreich. 132 pp. Braunschweig (Lithoprint ed. 1946. Ann Arbor, Mich.: Edwards Bros.). — Kramer, P. J. & K. M. Wilbur (1949) : Absorption of radioactive phosphorus by mycorrhizal roots of pine. Science 110:8-9. — Magrou, J. (1943) : Des orchidées à la pomme de terre. 203 pp. Paris. — Peyronel, B. (1939-40) : Luce, humus e micorrizìa. Atti d. R. Acc. Sc. di Torino 75:13 pp. — Schaede, R. (1948) : Die pflanzlichen Symbiosen. ed. 2. 187 pp. Jena.



CONTENTS

PREFACE vii

CONTENTS xi

LIST OF TEXT ILLUSTRATIONS AND PLATES xv

Lecture I

The Rise of Mycotrophic Study:- THE BEGINNING OF ROOT STUDY — EARLY STUDIES ON ROOT HAIRS — EARLY STUDY OF NODULES — NUTRITION OF MONOTROPA — NUTRITION OF ORCHIDS — MYCOTROPHY IN FERNS AND FERN ALLIES — MYCOTHALLISM IN LIVERWORTS — EARLIEST OBSERVATIONS OF MYCORRHIZAE — DISCOVERY OF THE HARTIG NET — NATURE OF MYCOTROPHISM — MYCORRHIZAE DEFINED — CONFLICTING CLAIMS OF DISCOVERY — FRANK'S ANTAGONISTS — BERNARD AND ORCHID SYMBIOSIS — TUBER FORMATION AND PERENNISM — SYMBIOSIS IN LOLIUM — OBLIGATE MYCOTROPHY IN HEATHS — IDENTITY OF THE MYCORRHIZAL FUNGI — ISOLATION OF MYCORRHIZAL FUNGI — THE NITROGEN THEORY OF MYCOTROPHISM — THE STAHLIAN HYPOTHESIS — CARBON HYPOTHESES — GROWTH PROMOTING SUBSTANCES — PHAGOCYTOSIS — PHYCOMYCETE MYCORRHIZAE — FOREST TREE MYCORRHIZAE — ECOLOGY OF MYCORRHIZAE — TRENDS 1

Lecture II

The Occurrence of Mycorrhizae:- REASONS FOR STUDYING MYCORRHIZAL OCCURRENCE — THE OCCURRENCE OF ROOT-HAIRS — GENERALITY OF MYCORRHIZAL OCCURRENCE — SYMBIOSIS AMONG ALGAE — MYCOTHALLI AMONG LIVERWORTS — FUNGAL SYMBIOSIS WITH PTERIDOPHYTES — SYMBIOTIC FUNGI AMONG ARTHROPHYTES — MYCORRHIZAE AND MYCOTHALLI OF LYCOPSIDA — GYMNOSPERMOUS MYCORRHIZAE — MYCORRHIZAE OF CYCADS — MYCORRHIZAE OF GINKGO — MYCORRHIZAE AMONGST THE TAXACEAE — MYCORRHIZAE IN PINACEAE — MYCORRHIZAE IN GNETACEAE — THE METHOD OF OPPORTUNISM — MYCORRHIZAE IN APETALAE 14

Lecture III

The Fungal Endophytes:- Nature of the mycorrhizal fungi — Phycomycete mycorrhizal fungi — Ascomycetous mycorrhizal fungi — Hemibasidiomycetes — Hymenomycetous mycorrhizal fungi — Gasteromycetous mycorrhizal fungi — Phallomycetous mycorrhizal fungi — Form genera of mycorrhizal fungi — Fusarium — Rhizoctonia — Phoma — Mycelium radicis — Conclusion 37

Lecture IV

Fossil Mycorrhizae:- Limitations of the fossil record — Sources of material — Fossil Phycomycetes — Fossil hepatics — Fossil ferns and their endophytes — Mycorrhiza of fossil Lycopod — Mycorrhizae in a seed fern — Mycorrhizae in Cordaites — Summary 47

Lecture V

Distribution of Mycotrophic Plants:- General — Germany — France and the Iberian Peninsula — British Isles — Lowlands and Scandinavia — Baltic and Russian States — The Arctic — The Alps — Central Europe — The Balkans — Italy — Africa — Asia — Java — Japan — New Zealand — Australia — South America — West Indies and Central America — North America — North-eastern U.S.A. — Southern U.S.A. — Central U.S.A. — Rocky Mountains — Pacific Coast 53

Lecture VI

Mycotrophic Plants and Their Environment:- Soil as a mycotrophic habitat — Mycorrhizae and soils — Humus — Mycorrhizae as soil indicators — Mycorrhizae in the soil profile — Soil texture — Soil moisture — Soil solution — Soil reaction — The use of free nitrogen — Soil temperature — Altitude — Light — Phenology — Mycorrhizae in relation to habitat — Salt marsh — Prairie — Soil Inoculation — Compost studies 68

Lecture VII

Mycothalli and Mycorrhizomes:- General character — Mycothalli in liverworts — Infection of mycothalli —

Limitation of endophyte — Digestion of endophyte — Mycothalli in fern gametophytes — Mycothalli in lycopod gametophytes — Mycorrhizomes in ferns — Mycorrhizomes in orchids — Mycocaryopses and infection of aerial organs 91

Lecture VIII

Mycodomatia:- Significance of the term — Psilotum — Cycads — Podocarpus — Casuarina — Myrica — Alnus — Polygonum — Raphanus — Tribulus — Legumes — Ailanthus — Ceanothus — Elaeagnus — Hippophaë — Coriaria — Eucalyptus — Daucus — Ericads — Solanum — Melampyrum — Orobanche — Composites — Juncus — Molinia — Cyperus — Asparagus — Allium — Orchids . . 103

Lecture IX

Structure of Mycorrhizae:- The kinds of mycorrhizae — Mycorrhizal compared with non-mycorrhizal roots — External form — Pseudomycorrhizae — The colours of mycorrhizae — The exterior surface — The mycorrhizal apex — Renewed growth — Ectotrophic vs. endotrophic — Ectotrophic mycorrhizae — Endotrophic mycorrhizae — Peloton mycorrhizae — Vesicular-arbuscular mycorrhizae — Arbuscles — Ericaceous mycorrhizae — Coralloid mycorrhizae — Tuberous mycorrhizae — Outer cortex and passage cells — Wall tubules — Hartig net — The stele 116

Lecture X

Obligate Symbiosis:- Fungi and trees — Ecological influences — Special cases — Fungi and herbs — Obligatism and nutrition — The ericads — The problem of Calluna — Other Ericaceae — The orchids — Germination of orchid seed — Necessity of orchid fungus — Degeneration of orchid fungus — Hydrolysis of starch — Sugars and asymbiotic germination — Fungus supplies sugar — Impotence of unaided small seeds — Carbon supply to embryo — Obligatism in lower plants — Summary 136

Lecture XI

Theories of Mycotrophy:- Contrasted concepts — Parasitism — Curbed pathogens — Schools of mycotrophism — Mycor-

RHIZA REPLACES ROOT-HAIRS — MYCOPHAGY — ROMELLIAN HYPOTHESIS — NITROGEN THEORY — CHEMICAL STUDIES OF THE NITROGEN THEORY — IMPORTANCE OF P — STAHLIAN HYPOTHESIS — HATCHIAN HYPOTHESIS — TRANSPIRATION AND MYCOTROPHY — OBJECTIONS TO THE STAHLIAN HYPOTHESIS — BJÖRKMAN'S HYPOTHESIS — CARBONACEOUS THEORIES — HYDROCARBON HYPOTHESIS — CARBOHYDRATE HYPOTHESIS — GROWTH-PROMOTING SUBSTANCES — LIMITATION OF MYCOTROPHIC HYPOTHESES — THE INTAKING MECHANISM — ECTOTROPHIC INTAKE — ROOT-HAIRS VERSUS SETAE — ENDOTROPHIC INTAKE — HYPHAE AS NUTRIENT CONVEYORS — TELEOLOGY IN MYCOTROPHISM . 148

Lecture XII

Mycotrophic Phagocytosis: SIGNIFICANCE OF THE TERM — OLDER DESCRIPTIONS OF PHAGOCYTOSIS — PHAGOCYTOSIS IN PELOTON MYCORRHIZAE — PHAGOCYTOSIS IN ARBUSCULAR-VESICULAR MYCORRHIZAE — PHAGOCYTOSIS IN ERICACEOUS MYCORRHIZAE — PHAGOCYTOSIS IN ECTOTROPHIC MYCORRHIZAE — LIMITATION OF ENDOPHYTE — LIMITATION IN ORCHIDS AND OTHER HERBS — LIMITATION IN HEPATICS — LIMITATION IN ROOT APICES — LIMITATION IN LONG ROOTS — LIMITATION IN GREEN TISSUES — SUMMARY OF LIMITATION — THE STARCH RELATION — CONCLUSION 164

Bibliography 183

Subject Index 216

Index of Plant Names 219

LIST of TEXT ILLUSTRATIONS and PLATES

FRONTISPIECE. — The development of *Odontoglossum* iv

FIG. 1. — Longitudinal section through mycothallus of *Pellia epiphylla* .. 18

FIG. 2. — Mycorrhizae in *Pinus virginiana* 27

FIG. 3. — Mycorrhizae in Sugar Maple, *Acer saccharum* 32

FIG. 4. — Renewed growth of mycorrhiza-bearing mother-root of *Pinus Strobus* 82

FIG. 5. — Section through an older mycothallus of *Botrychium obliquum* 94

FIG. 6. — Portion of mycothallus of *Lycopodium obscurum* shown in section 96

FIG. 7. — Longitudinal section through apex of a mycorrhiza of *Pinus rigida* 120

FIG. 8. — Renewed growth of a mycorrhiza of *Pinus virginiana* 122

FIG. 9. — Cross-section of an ectotrophic mycorrhiza of *Quercus montana* 124

FIG. 10. — Cross-section of endotrophic mycorrhiza of *Acer Negundo* 126

FIG. 11. — Section of a mycorrhiza of *Abies balsamea* 133

FIG. 12. — Portion of a cross-section of the ectendotrophic mycorrhiza of *Cornus florida* 166

FIG. 13. — Portion of a section through mycorrhiza of *Abies balsamea* 168

FIG. 14. — A cell from mycorrhiza of *Allium sphaerocephalus* 170

FIG. 15. — Portion of a longitudinal section through a mycorrhiza of *Pteridium aquilinum* 171

FIG. 16. — Some cells from mycorrhizal cortex of *Fraxinus americana* ... 173

PL. 1. — One of the earliest illustrations of mycotrophic infection, by EDOUARD PRILLIEUX, 1856 209

PL. 2. — Mycorrhizae in Scot's Pine, *Pinus sylvestris* 210

PL. 3. — Mycorrhizae of *Carpinus betulus* and *Fagus sylvatica* 211

PL. 4. — Effect of mycorrhizae on plant growth 213

PL. 5. — Photomicrograph of a portion of a fossil mycorrhizome of *Scleropteris illinoiensis* 215

"Das Wort Symbiose bedeutet zunächst ganz allgemein das regelmässige Zusammenvorkommen von Lebenswesen unter denselben äussern Faktoren. . . . . Die beiden Organismen bilden nach der Vereinigung einen neuen Organismus, der als einheitlich zu betrachten ist und unter neuen Bedingungen den Kampf ums Dasein aufnimmt." (H. Burgeff, 1909).

" . . . der Pilz, als der alleinige Zuführer alles für den Baum erforderlichen Wassers und Nährmaterials aus dem Boden erscheint." (A. B. Frank, 1885).

" . . . the similar (tropical) conditions that prevailed during the Carboniferous mitigate our surprise at finding symbiosis occurring so far back as this." (E. W. Berry, 1904).

"Mit Anwesenheit oder Abwesenheit von Baumhumus die Mycorhiza entsteht oder verschwindet." (A. B. Frank, 1888).

" . . . the host plant may, as it were, gain the upper hand and cause the fungus to enter into a mutually beneficial partnership." (Cavers, 1903).

"Quoique l'existence pour ainsi dire normale d'un parasite dans les tissus d'une plante soit un fait très singulier, on peut observer assez fréquemment dans la famille des Orchidées." (E. Prillieux, 1856).

"On any hypothesis, the evolution of an obligate relation with a parasitic or facultatively parasitic fungus is difficult to explain." (M. C. Rayner, 1927).

" . . . ce me semble, inférer que la matière brune sert à l'alimentation de la plante. . . " (E. Prillieux, 1856).

"La symbiose est à la frontière de la maladie." (N. Bernard, 1909).

Lecture I

THE RISE OF MYCOTROPHIC STUDY

The Beginning of Root Study:—After Herbalists had done their work and, by means of wood-cut and description, had made known the flora of Europe, inquiry began to be made into physiology of plants. It is said that MAJOR, in 1665, directed attention to circulation of sap. Four years later, RAY proved ascent and descent of sap and its lateral movement in certain plants. At the same time, WOODWARD demonstrated by experiment that roots take in, not merely water, but also materials dissolved in the water. Both RAY and WOODWARD published their papers in the Philosophical Transactions of the Royal Society. Beginning in this manner, with study of roots in water culture, plant physiology was turned to root-hair study, from which it has not seriously deviated to this day.

Early Studies on Root Hairs:—Early observers were perhaps influenced by knowledge of circulation of blood in animal bodies and were doubtless expecting to find vessels in plants. When MALPIGHI found root-hairs on elm, black poplar, and willow roots, he assumed that these structures took up crude sap and passed it on to vessels. GREW, publishing about the same time (1682) had decided that spongy ends of roots served admirably for absorption of water and food from the soil; and HALES in 1727 and DE LA BAISSE in 1733 tried to show experimentally that the greater quantity of water used by the plant was taken up through ends of the root-tips and that root-hairs were only incidental phenomena. To these hypotheses was added in 1768 that of S. SIMON, who stated that roots, at least the noduliferous, are merely excretionary organs which serve to eliminate excess elaborated sap from the plant. In the earliest years of the nineteenth century, GARRADORI showed that root-hairs are wanting in water, from which fact he concluded that root hairs serve for absorption of moisture from the air and not for absorption of liquid water, which, he concluded, must be taken up by the spongy ends of roots. But, according to MOLDENHAWER, root-hairs may be compared to druse-hairs of leaves: they secrete a liquid which serves

to dissolve food materials somewhat as saliva does in animals. It was F. MEYEN, in 1838, who came to the modern view that root-hairs serve merely to increase the outer surface area of the root.

By such studies attention was focused on root-hairs until Botany was firmly moulded to the view that higher plants are nourished by a root-hair mechanism. So positive had Botany become that by 1883, FRANK SCHWARZ (from whom we have quoted much of the preceding paragraph) was able to state without exciting contradiction: "From my researches it may be stated that root-hairs are present on most plants, and when a plant fails to produce root-hairs it may be counted an exception." He listed as exceptions: water and swamp plants, and those the water and salt requirements of which are met in a special way, as in conifers, noduliferous plants and in part by parasites.

Early Study of Nodules:—Thus it was, not by extended observation or study of plants in nature but by sheer dogmatism that root-hairs came to be regarded as the predominate root structures of higher plants. Hairless roots were considered exceptional, but they were constantly being noted. MALPIGHI, early microscopist that he was, had described and figured nodules while DU HAMEL DU MONCEAU in 1758 had stated that such structures were generally found on leguminous plants. Even MEYEN, in 1829, who has been accredited with discovery of mycorrhizae, simply described nodules of the alder. Alder nodules were more carefully studied by WORONIN in 1867 but his inadequate facilities led him to confuse bacterial strands with fungal hyphae. Even to this day there is confused thought about root-nodules for some investigators assert them to be purely bacterial while others consider them fungal.

Nutrition of Monotropa:—Besides nodulous roots there were obviously other exceptional kinds. The waxy *Monotropas* that appear in deciduous woods have no apparent root-hairs and were long considered parasites. It was thought that they must be attached to tree roots for they always grew under the trees and in a thick mat of humus and intertangled rootlets, although as early as 1832 FRIES had noticed a fungus connected to *Monotropa*. Several investigators reported on it in that short-lived journal, The Phytologist, and came to the conclusion that, whatever else this plant might be, it was certainly not a parasite. One of the observers, LUXFORD, in 1844,

hazarded the suggestion that *Monotropa* secures nutriment from the surrounding humus. DRUDE, in 1873, conformed to tradition by stating that *Monotropa* starts life as a parasite but later, he asserted, the plant becomes a saprophyte on soil humus, a fungus being present in its tissues. The true nature of *Monotropa* was first made clear by KAMIENSKI in 1881, who carefully described the structure of its mycorrhiza and indicated that the plant is supplied with nutriment by a fungus which derives its materials from the soil humus. His papers (for there were two) have long since become forgotten history but they were considered important in their day.

Nutrition of Orchids:—Orchids, like *Monotropas,* also proved exceptional in their root structures. Indeed, many orchids have no roots! In place of roots they have branched stems that form coral-structures that anchor the plant in the rich humus soil in which it grows. It was in 1842 that SCHLEIDEN described what were later recognized as fungal hyphae in *Neottia* (for *Neottia* has been as necessary to orchid students as *Drosophila* to geneticists), but SCHLEIDEN confessed he did not know what the "tubes" were which he had observed in the rhizomes. Unless, he said, they were like the ones which GÖTTSCHE had found in liverworts. Five years later REISSEK identified true fungal hyphae in rhizomes of many orchids but he oddly concluded that these hyphae developed from starch grains. But SCHACHT in 1854 showed that the starch in reality was utilized by the fungus, which forms a weft of hyphae about the starch grains. Just how the starch was digested (by the process we now call phagocytosis) was described by PRILLIEUX in an excellent paper that appeared in 1856. He found in orchid cortical cells (needless to say, of *Neottia*), a yellowish-brown matter, and he noted that these cells retained their nuceli which were of great size and provided with two nucleoli. The matter seemed to be nitrogenous and was woven about with septate hyphae but as phagocytosis proceeded the matter dwindled; and PRILLIEUX concluded that this matter served as nutriment for the orchid. He observed, too, that cells filled with granular matter at flowering time gradually lost the matter as anthesis advanced. The granules were apparently absorbed and they probably nourished the orchid. But PRILLIEUX's work was little regarded and later papers by other authors were farther from the truth. Thus REINKE in 1873 suggested that this yellowish matter which he called slime, acted as a pumping organ, swelling up as water was taken in

and forcing the water on through the tissues. MOLLBERG in 1884 questioned whether the fungal endophyte brought any nutriment to the orchid; while EIDAM in 1879 attempted to culture the fungus by allowing fungi to develop on orchid "roots" placed in damp air.

Mycotrophy in Ferns and Fern Allies:—A yellow matter similar to that found in orchids was found in lycopods by VAN TIEGHEM in 1871. He described this substance, and it was further described by BRUCHMANN in 1874, who found it free from starch. BRUCHMANN noticed, too, that in older tissues the fats and nitrogenous substances dwindled in amount while a quantity of chrome-yellow granules appeared. Ten years later (*cf.* TREUB, 1884) it had become established that endophytes are generally distributed in lycopods and that their presence is not harmful. Indeed, TREUB regarded them as commensals.

In ferns, especially the *Ophioglossaceae* and *Marattiaceae,* fungal infection had been reported, and also presence of yellow matter. Since the infection was present in fern stems, these structures were appropriately termed fungal-rhizomes or mycorrhizomes. The term mycorrhizome was coined by DANGEARD in 1891, in his study of *Tmesipteris.*

Mycothallism in Liverworts:—In various liverworts a foreign substance was observed in the form of large brown cask-shaped structures (MILDE, 1851), the significance of which could not be discerned. But GÖTTSCHE in 1843, as earlier stated, had observed a system of branching tubes in *Aneura;* and these tubes were definitely described as hyphae by LEITGEB in 1874. Since the thallus of the liverwort harbours a fungal endophyte, it is known as mycothallus.

Earliest Observations of Mycorrhizae:—The association of fungi with roots of higher plants has long been known. In THEOPHRASTUS' Enquiry Into Plants (according to HORT's translation) we read: "For as for the fungi which grow from the (oak) roots or beside them, these occur also in other trees." THEOPHRASTUS (or TYRTAMUS, to use his proper name) may have been walking in a woodland and observed sporophores of fungi which he seems to have traced to tree roots. It is a long step from the third century B.C. to A.D. 1829, but the next reference to mycorrhizae was made in the latter year by MEYEN. In a short paper he called attention to peculiar structures which he found on beech roots, which he believed were beginnings of parasitic plants. Perhaps they were mycorrhizae, perhaps not. But attention was being directed to root structures and little by little knowledge increased. In 1837 LINK stated that most

roots are formed in humus; TULASNE in 1841, that tree roots were found frequently surrounded by mycelium of the truffles fungus; while GASPARINI in 1856 stated that a fungal mantle was found about roots of chestnut, hazel and pine. Hairs were considered so inevitably present on roots that it was heresy to speak of anything else, hence SCHACHT in 1860 cautiously stated that, while root-hairs are present on such trees as oak and beech, they were less abundant on pine and fir. SCHWARZ, from whom we have already quoted, presented a list of conifers from which they were lacking.

Discovery of the Hartig Net:—When coniferous rootlets were examined in section it was seen that the wall possessed what was termed a peculiar cell-wall thickening; and it is an odd fact that morphologists beheld fungal mycelium in such rootlets for a long time without being aware of its nature. Thus, NICOLAI in 1865 gave a tolerable description of what is now known as Hartig net without realizing that he was describing a foreign organism in the conifer. VAN TIEGHEM in 1871 also described these "thickening bands"; and he noticed furthermore that the penultimate layer of root cortical cells was filled with a solid deposit, a fact of significance in mycotrophic nutrition of these conifers. It remained for REINKE in 1873 to call attention to the similarity of these supposed thickenings to mycelial strands which GÖTTSCHE had found in the liverwort, *Pellia*. With realization that tree rootlets were characteristically invaded by hyphae, specific infections were described. Thus, BOUDIER in 1876, described *Elaphomyces* on birch, oak and chestnut roots; and he noticed furthermore that such roots were found in acid but not in alkaline soils.

Nature of Mycotrophism:—The nature of the fungus-host relation was next considered. That the fungus in the root was a harmless parasite was the opinion of RESA, expressed in 1878; and GIBELLI in 1873 had the same opinion. But REESS, publishing in 1880, questioned whether the fungus was a parasite on the tree-root or a saprophyte on soil humus. The supposed parasitism of some plants which live in rich humus, such as the *Burmannias*, had been questioned by CRÜGER in 1848; and clear recognition of saprophytes as distinct from parasites had been made by SOLMS-LAUBACH in 1868. By a shrewd guess, PFEFFER in 1877 came to the conclusion that saprophytes actually make use of the materials of humus. Without experimental evidence, he inferred that mycorrhizal fungi obtained nutrient material from the humus and transferred it to the host plant which, of itself, was incapable of utilizing the otherwise un-

available materials of the humus. At the same time PFEFFER realized that the fungus was essentially a parasite which was no more than kept in check by the host plant. Since the picture of mycotrophy presented by PFEFFER is so close to the actual phenomenon, he may perhaps be considered *the true discoverer of the mycorrhiza.*

Mycorrhizae Defined:—But, important as PFEFFER's paper now appears, it attracted little attention and it was not until 1885 that world-wide attention was suddenly drawn to fungus-roots. Just why ALBERT BERNHARD FRANK's dissertation "Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze" should have had such a profound effect is for others to determine: Suffice to say, modern mycorrhizal study dates from this paper. In it, FRANK had invented and defined the term in these words: "Der ganze Körper ist also weder Baumwurzel noch Pilz allein, sondern ähnlich der Thallus der Flechten, eine Vereinigung zweier verschiedener Wesen zu einem einheitlichen morphologischen Organ, welches vielleicht passend als Pilzwurzel, Mycorhiza, bezeichnet werden kann." As the word comes from the Greek and good usage requires doubling the letter r in compounding, we now write it, mycorrhiza.

Conflicting Claims of Discovery:—Immediately after publication of FRANK's epochal work there was a rather entertaining flurry of papers. Some people wished to call attention to their own work, published earlier than FRANK's, while others desired the world to know they had often seen exactly what FRANK had described. Several pressed the claims of KAMIENSKI as the discoverer of the mycorrhiza, but as we read his 1884 paper we find only this vague statement: "I suppose, moreover, without being able to confirm it, that the fungus which grows on *Monotropa* is the same which lives parasitically on roots of conifers and other trees. This fungus deforms the root and occasions their dichotomy. I have found indeed, among the roots of *Monotropa*, a great quantity of other roots which were very fine, deformed, and belonging to trees which grew all there about: they were so interlaced that the mycelium which webbed them on touching might be said to be interblended."

Frank's Antagonists:—Then, too, there was a persistent effort to label mycorrhizal fungi as mere harmless parasites like the leaf-spot fungi. ROBERT HARTIG was a particularly active opponent of FRANK; and HARTIG's views were held in later years by the American, MCDOUGALL. FRANK spent little time in advancing mycorrhizal

study for he turned to other studies, but he returned to the defence of his hypothesis of mycotrophy from time to time. At first he thought that mycorrhizal fungi are concerned especially with nitrogen nutrition of the higher plant, bringing nitrogen salts into the mycorrhiza. But later he taught that the higher plant is actually a parasite on the fungus, drawing it into the root, tending and finally devouring it.

Bernard and Orchid Symbiosis:—At the turn of the century a new phase of mycotrophic study was developed. Hitherto the interest had been chiefly with forest plants, following the initiative of FRANK. But when NOËL BERNARD began to publish on mycotrophism of orchids an important new branch of science was opened up. BERNARD isolated from orchid tubers fungi which he classified into three groups and placed in the genus *Rhizoctonia.* These fungi, he found, were able to cause orchid seed germination and, lacking presence of a fungus, there was no germination. It is interesting to note that SALISBURY, when he described orchid seed germination in 1804, failed to note presence of fungus. But BERNARD did not assert that a fungal symbiosis was an inevitable necessity, for he induced asymbiotic germination of orchid seed with salep, a polysaccharide derived from dried orchid tubers. Sterile cultures were grown by KNUDSON, who showed that in greenhouse propagation of orchids sugars may be used in germination of the seeds in place of fungi; but in nature it is of course the fungus that is responsible for the germination.

Even before BERNARD's untimely death in 1911, BURGEFF had been publishing on orchid mycotrophism. He called the endophytes *Orcheomyces,* but later made use of the less convenient designation of *Mycelium radicis.*

Tuber Formation and Perennism:—BERNARD was convinced that tuber formation in orchids and other plants was due to their symbiotic life with fungi, and that after many generations of such symbiotic life the "habit" of forming tubers was acquired so that tubers were still formed by the plant even in the absence of fungal aid. Thus the potato, native of Andean highlands, formed tubers in the Andes in symbiosis with a species of *Phoma,* but in northern latitudes, as in France, tubers were still formed without presence of a fungus. He advanced the idea that influence of a cold climate parallels the action of the fungus, and pointed out that in a hot climate such as that of Algiers, tuber formation "degenerated" and tubers were no longer formed in absence of a fungus. Using this

principle, BERNARD's successor, MAGROU, and his compatriot, COSTANTIN, have developed an hypothesis of perennism by which they attempt to account for existence of perennial plants on a symbiotic basis.

Symbiosis in Lolium:—Another phase of mycotrophic study dealt with fungal infection of seeds, particularly of the seed-fruits or caryopses of *Lolium,* a genus of grasses. Discovered by VOGL in 1897, the constant presence of hyphae in the hyaline layer of the caryopsis and typical stages of digestion, are curious phenomena that have nevertheless been established by repeated researches, especially at the hands of MCLENNAN in Australia. The endophyte also occurs, according to NEILL (1940) in leaves of *Lolium* but not in those of other pasture grasses.

Obligate Mycotrophy in Heaths:—Infection of seeds of heaths, especially of *Calluna,* has likewise been reported, particularly by RAYNER; indeed, practically all tissues of the plant were said to be invaded by the otherwise mycotrophic fungus. RAYNER claimed that mycelium present in the seed-coat of *Calluna* grows into the developing plantlet so that a sterile culture of this heath can not be obtained. This assertion has been challenged by KNUDSON and by FREISLEBEN. According to FREISLEBEN (1935), the fungus causes an amelioration of soil conditions in which the heath grows and does not directly affect life of the heath.

Identity of the Mycorrhizal Fungi:—The specific identity of mycotrophic fungi has proved more puzzling than might at first be suspected. It would seem that sporophores directly attached to tree roots might be safely considered the mycorrhizal fungi of these hosts. Constant association of truffles with tree roots furnished the incentive for FRANK's study of the mycorrhiza, and there are many other such associations, as, for example, *Hebeloma* with birch. In many cases rhizomorphs have been traced from sporophore to mycorrhiza and the associated fungus has been termed mycorrhizal, but such associations may actually be the result of a secondary infection. Hence the numerous citations of mycorrhizal fungi based on connection of sporophore with mycorrhiza are not necessarily valid.

Isolation of Mycorrhizal Fungi:—Isolating the fungus directly from the mycotrophic organ has proved, in most cases, impracticable; and the usual method of identifying the fungus in question is to grow a suspected fungus in pure culture, inoculate it into a sterile

seedling and if a mycorrhiza results, to consider the fungus mycorrhizal. The pioneer in synthesizing mycorrhizae was Josef Fuchs, and one of the most prominent investigators of such syntheses is Melin. Still more recently, Modess has reported many such syntheses; and Fries has formed synthetic mycorrhizae with monospore mycelia.

The Nitrogen Theory of Mycotrophism:—As to the nature of the mycorrhizal symbiosis, numerous hypotheses have been advanced. It has already been noted that R. Hartig and others considered the symbiosis to be a case of harmless parasitism, and that Kamienski maintained that there is a beneficial symbiosis only in the case of *Monotropa*. Frank had concluded that the parasitism was just the reverse,—that the higher plant was a parasite on the fungus! He had found that "the tissues of a mycorrhizal tree are nitrate free". As it was known that the fungus could readily make use of ammonia and organic nitrogen compounds, he considered it self-evident that in this way such compounds were taken up from nitrate-free or nitrate-poor soils and liberated in the mycorrhiza. The nitrogen nutrition hypothesis which Frank originated was elaborated by subsequent investigators, von Tubeuf, Möller, Müller, Weiss, to mention a few; and especially by Melin, who has conducted many researches into the nature of mycorrhizae of forest trees. Melin appears to consider that, in Swedish forests, the problem of the mycorrhiza is above all a nitrogen problem.

The Stahlian Hypothesis:—On the other hand, Stahl in 1900 emphasized the mycorrhizal intake of all minerals used by the higher plant. Trees being brought into competition with fungi and bacteria for nutrient materials contained in the soil are seriously limited in their food supplies; and in the mycorrhizal fungi find provisioners that bring water and dissolved salts into the roots. It is not merely nitrogen that is brought into the root, according to this hypothesis, but all the minerals the soil solution contains. As a corollary the supposed relation of transpiration to mycotrophy was pointed out. Plants possessing a large transpiration stream bring a considerable quantity of mineral salts into the higher plant and deposit them in its tissues. Plants with a smaller transpiration stream have a smaller salt intake and are presumably compensated with the salts provided by the mycorrhizae. But the Stahlian hypothesis ran into difficulties which are detailed later; and it met with little favour until supposedly revived by Hatch in 1937. In reality Hatch originated a new hypothesis which states among other things that

mycorrhizae greatly increase the absorbing surface of the rootlet. The Hatchian hypothesis quietly drops the Stahlian conception of transpiration streams. Still more recently, in 1943, ROUTIEN and DAWSON stated that "mycorrhizae increase the salt-absorbing capacity of the roots primarily by adding to the supply of exchangeable hydrogen-ion derived in part at least from carbonic acid."

Carbon Hypotheses:—Besides the nitrogen- and mineral-nutrition hypotheses, there are carbon hypotheses which have been advanced by two investigators. MCLENNAN in 1926 stated that the more generally accepted ideas connecting mycotrophism with nitrogen nutrition were insufficiently founded; and she concluded on the basis of her researches on *Lolium* that "a metabolic exchange takes place from the fungus to the higher plant, with the result that the later obtains a supply of fat or oil." MCLENNAN believed that the researches of KNUDSON and of BERNARD lent support to this conception. Another carbon hypothesis was advanced by YOUNG in 1940, in these words: "It is thought that as well as providing a more efficient absorptive system on the tree roots so that mineral salts and nitrogen compounds are more readily available, the mycorrhizas also furnish a means of augmenting the carbohydrate supply . . . In the author's conception the fungus manufactures its own carbohydrate supply from the available soil organic matter, and a portion of this is transferred to the higher plant by means of the intimate association existing in the mycorrhizal structures."

Growth Promoting Substances:—Complex substances of the humus suggested still further hypotheses. LINK, WILCOX and LINK in 1937 had suggested that "heteroauxin applied to a plant can either substitute in part for its autoauxones or augment their action, and the well-known fact that soil fungi and bacteria produce heterauxin suggests that some of the beneficial effects of humus soil may be due to the auxones of decaying plant debris or soil flora." Still more specifically, MAGROU in 1939 reported a more luxuriant development in *Arum* as a result of supplying it with aneurin (Vitamin B_1). MELIN in 1939 and 1940 found increased growth in seven mycorrhizal fungi when these organisms were given aneurin or yeast filtrate. In mycorrhizae of Monterey pine, MACDOUGAL and DUFRENOY reported in 1943 that the "independent growth of isolated segments of mycorrhizal roots makes it obvious that through these hyphal branches the root receives from the soil not only the C, N, O, P necessary to build up the nucleus, the cytoplasm and its inclusions (mitochondria and plastids), cell-walls, but also the mineral com-

pounds ordinarily taken in by root-hairs, and growth promoting substances, thiamin, nicotinic acid . . ."

Phagocytosis:—Whatever may be finally decided as to the nature of the mycorrhizal symbiosis, it is observed that something is released in the tissues of the higher symbiont by the fungus. It is becoming increasingly evident that in what are termed "digestion cells" of the host the hyphae break down and disgorge their contents, the matter being digested and assimilated by the host. Since it is a cellular digestion, BERNARD called it a phagocytosis; but the whole process had been pictured and to some extent described long before BERNARD. In 1943 it was suggested by KELLEY that the whole mycotrophic relation depends on a balancing of ionic concentrations between fungus and higher plant.

Phycomycete Mycorrhizae:—While the majority of mycorrhizal fungi are basidiomycetes, it is now recognized that in many mycotrophic symbioses the fungus is a phycomycete. Such symbioses were studied in earlier years especially by PEYRONEL. RAYNER, in 1935, commented on the "remarkably wide geographical distribution of this 'Phycomycete type' of mycorrhizal association, its prevalence in plant species of the most diverse affinities, together with its recorded appearance in certain crop plants. . ." Other more recent studies of the phycomycete mycorrhizae include those of BIRAGHI in 1936, on cereal grains, BAIN in 1937 on cranberry, SABET in 1939 on cotton, and RUGGIERI in 1937 on *Amygdalus*. BUTLER in 1939 presented a paper summarizing what was then known of this sort of mycorrhiza, terming it the vesicular-arbuscular mycorrhiza and grouping the endophytes under the generic name of *Rhizophagus*.

Forest Tree Mycorrhizae:—Although so much work has been done on forest tree mycorrhizae, knowledge concerning them is still defective. Considerable is known of those that occur on pine, spruce, larch, beech and some others, but researches upon them have been done by a few individuals working with limited material. The extent of mycorrhizal occurrence, both taxonomic and geographical, is still a matter of conjecture and, as most of mycorrhizal research has been done in Europe, the forests of the other continents are for the most part still uninvestigated by students of this science. Moreover, the natural difficulties in the way of isolating the mycorrhizal fungi make for ignorance of the symbiosis, for the syntheses of seedlings and fungi in pure cultures show merely what man can achieve and not what occurs naturally in the forest. Then, too, the nutrition of

forest trees is largely conjectural. From information now extant one cannot say with accuracy whether trees have root-hairs or mycorrhizae, much less the exact mode of nutrition of any particular kind of tree. Even were it concluded that the tree was nourished with the aid of mycorrhizae, the precise nature of mycotrophism is still in doubt. Hence the foresters' dealings with trees are akin to the mediaeval doctor's treatment of his patients.

Ecology of Mycorrhizae*:—The earlier mycotrophic problems in ecology involved the relation of mycorrhizae to sandy soils and to humus. More recently there has been discussion regarding the presence or absence of mycorrhizae in prairie soils with reference to establishment of trees on such areas. It has been claimed that the endophytes are absent from prairie soils and that such soils must be inoculated with suitable fungi before mycorrhizae will be formed. But investigators have not been careful to distinguish between prairie soils and the more arid steppe soils. Then, too, several investigators report abundant endophytes in these soils, and recent observations show that there is a rapid spread of trees into certain prairie areas.

As to soil reaction, there is more nearly an unanimity of opinion, for it is evident that mycorrhizal fungi thrive best in acid media. Hence, mycotrophic structures are more likely to be found in acid soils while root-hairs may be expected in mull soils. But in regard to soil solution, little can be said, for in spite of the number of investigations into nutritional and soil problems and the multiplicity of papers on salts in soil solution, the actual connection of the plant with the soil has been almost completely ignored. To say that a plant has mycorrhizae and is nourished by mycotrophy has been regarded as sufficient, and whether materials get from the soil into the plant by mechanical means or by black magic is left to the imagination of the reader. All the detailed studies of soil solution in the B horizon have no necessary connection with a large proportion of plants in native habitats. And thus the mechanism for the intake of materials into mycorrhizae is a subject of research awaiting investigation. Yet some attention has been paid to the microhabitat of the fungus-root and its community of organisms, the rhizosphere as it has been appropriately called.

*A valuable paper on the ecology of ectotrophic mycorrhizae, by Dr. J. L. Harley, of Oxford, has recently appeared in *Biological Reviews*.

Trends:—Early interest in mycotrophic study was taxonomic, and a sufficient number of plants was examined to show that the mycotrophic habit was widespread in the plant kingdom. Then there were sporadic collections which showed that mycotrophy existed in each of the continents. Today biology inclines toward philosophical dissertations which place the writer in an impregnable position since if anyone demurs it rests with him to disprove the postulates. Mycotrophic study still requires much work in taxonomy and morphology.

Lecture II

THE OCCURRENCE OF MYCORRHIZAE

Reasons for Studying Mycorrhizal Occurrence:—The occurrence of mycorrhizae is a subject of the first importance in mycorrhizal study. It is important for two reasons: first, to determine the *extent* of mycorrhizal occurrence, and, second, to determine mycorrhizal *importance*. It must be confessed that little is known of the extent of mycorrhizal occurrence. As we examine the history of the subject it is evident that human interest in mycorrhizae has followed a usual pattern: first there has been a flurry of interest, then an haphazard and eager collecting of various material from casual sources, and finally a settling down to solving problems of isolated detail that may or may not be important to the subject as a whole.

It would seem to be more logical to examine first of all the plant kingdom to determine whether mycorrhizae actually occur widely in that kingdom. We assume that they do but the assumption is not based on research. Two or three dozen investigators have gone into the woods and fields, they have sunk their digging tools into the earth, and whatever came up was made the subject of study. There are a few good papers on mycorrhizal occurrence, but very few. The classic paper is JANSE's account of the mycorrhizae occurring about Buitenzorg in Java, and after half a century we still consult the paper. JANSE consciously limited his research to chosen representatives of various plant families and as a *preliminary* study it is excellent, but it should be followed by similar papers on other members of the same, and of other, families.

In consequence of the fortuitous method which has hitherto been employed in mycorrhizal study, we have the following summary of what is now on record: Considerable good work on some of the hepatics; nothing on mosses or arthrophytes; a little on pteridophytes; good studies on the gametophyte generation of some lycopods; a very unequal emphasis on members of the Gymnospermae, with most members unknown as to mycorrhizal condition (all attention must be devoted to a few pines and spruce!); and a scattering of information about some angiosperms. On this slender evidence scientists assert the importance of mycorrhizae. They may be important and probably are important; but it is scarcely scientific to jump at conclusions. We

would be in a much better position to go forward with research if our research were founded on a considerable number of papers like that of JANSE's, or even such simple lists as that of KLEČKA and VUKOLOV (1935).

But if we know little of mycorrhizal occurrence, we know even less of root-hair occurrence. Morphologists have never paid much attention to roots except to study the vascular systems of older roots. Roots are in the ground, it takes considerable work to get them out, and the botanist pulls off a twig or a leaf or a flower and goes onward. An herbarium in which a "specimen" always included the root would be a curiosity. A thorough-going study of root structure has yet to be made, especially of the "absorbing" system of smaller rootlets: we still await a Systematic Morphology of Root-endings.

This statement leads to a consideration of the second reason for studying occurrence of mycorrhizae, namely, their importance. It is obvious that plants take in their nutrient materials through root-hairs or mycorrhizae except for a comparatively small number that are able to live without either. If root-hairs predominate in nature, then physiological research should be directed chiefly to root-hair plants; but if mycorrhizae predominate, then plant physiology should be concerned chiefly with mycorrhizae. Botany will some day be forced to a decision in the matter. At present botanists are in a position of ignorance, for they do not know what sort of root endings exist on the majority of plants in their natural haunts. They assume that root-hairs are the usual organs for intake of nutrient materials into plants, but their assumption cannot be substantiated from the records of research. Moreover, there is little prospect that research will be done on such structures, for the ruling motive in botanical science today appears to be a subservience to the authority of tradition.

The Occurrence of Root-Hairs:—According to FRANK SCHWARZ (1883), the first mention of root-hairs is found in MALPIGHI's Opera Omnia (1681), having observed them in elm, black poplar, and willow and he believed that in their tiny tubes he had before him that in which crude sap ascended and was later led into the vessels. He found them especially in those places where earth was not immediately in contact with roots. When the root hairs then pushed out into neighbouring soil, they grew around individual soil particles and surrounded them, so that they formed a span between the roots and soil particles. A similar clinging of the hairs was described by MALPIGHI in the roots of ivy. Almost simultaneously N. GREW (1682), in his "Anatomy of plants", advanced the idea that the spongy ends of roots served admirably for provision with food and

water; while HALES (1727), no less than the Father of Plant Physiology!, decided that root-hairs are only incidental phenomena in intake of materials from the soil, the chief intake being through the root tips. Among the seemingly countless authors of the first 20 years of the 19th century (yet producing nothing new), may be mentioned GARRADORI and MOLDENHAWER. The former noted that root-hairs are wanting in water, from which he concluded that they serve, not for absorption of liquid water, but moisture from the air, while liquid water is taken up by spongy root-ends. According to MOLDENHAWER, root-hairs may be compared to druse-hairs of leaves: they secrete a liquid which serves to dissolve the food materials, being comparable in a way to saliva of animals.

The first description that was given right direction was by F. MEYEN (*in* Neues System der Pflanzenphysiologie, 1838. Bd. 2, p. 6), who proceeded from a description of absorptive hairs of moss and characeous plants, showing incidentally that in these cases the root-hairs completely take the place of roots. He called attention furthermore to the supposed universal distribution of root-hairs in higher plants, investigated their development, and what is more important, attributed to them the direct intake of liquid water. He came to the view that root-hairs serve merely to increase the outer surface of the root, and he showed that the number of root-hairs formed is dependent upon external conditions. Next we may cite the work of G. GASPARRINI, the "Richerche sulla natura dei succiatori e la escrizione delle radici" (1856), which is a most comprehensive work on root-hairs but it offers in general nothing new. GASPARRINI had investigated quite a large number of plants and found them with few exceptions to have root-hairs; he did not go into a study of the conditions of root-hair formation but satisfied himself with describing their form, content, etc. The finest portions of earth, embedded in a gummy sort of a mass which clung to the root-hairs he considered to be excretionary products of the hairs. He even designated as such roots which had an evident root-cap. SCHACHT incorporated GASPARRINI's work in his text of 1859. Much more precise than his predecessors, SACHS (1860) made clear the significance and function of root-hairs; and from him the more modern phases of such study may be dated.

Generality of Mycorrhizal Occurrence:—In spite of our comparative ignorance of root structure in particular, it is known that in all major groups in the plant kingdom there are fungi living with other plants in mutualistic relation. No major group from "Thallophyte" to Spermatophyte is excepted. In the lower groups we speak of mycothalli or lichen bodies while in higher groups are mycorrhizo-

mata or mycorrhizae, but in all cases the relationship appears essentially the same.

Symbiosis among Algae:—Mutualistic symbiosis of fungi with algae, so far as known, is confined to lichens. While it is still maintained by some people that the lichen symbiosis is a parasitism of the fungus upon the alga, the balance of favour is with the Schwendenerian theory of mutualism. The algae, principally Cyanophytes and protococcid Chlorophytes, supply organic material, presumably sugary carbohydrates, to the fungi which take in water and dissolved salts from the exterior into the lichen body and thus to the enmeshed algae.

The lichen body is a thallus but it differs radically from the mycothallus of the liverwort, which is a tissue containing hyphal strands. In the lichen thallus the chlorophyll-bearing thalloid cells (algae) are discrete or loosely massed together (gonidia), not forming a tissue as in the liverwort; and the lichen thallus is for the most part a specially and characteristically formed mycelium. Then, too, the fungal symbiont of the lichen thallus produces reproductive structures (spores and soredia) while in a true mycothallus the fungus does not produce reproductive bodies.

Mycothalli among Liverworts:—Widespread occurrence of fungal symbiosis amongst liverworts has been demonstrated by the twenty-six investigators who, in the course of history, have studied mycothalli. It is to be assumed that anything so lacking in obvious utilitarian interest as a liverwort should attract but little general regard. It was apparently Schleiden who in 1839 first described what we know as fungal infection of a liverwort (*Pellia*),—not Leitgeb (1879) as has been erroneously stated; but as Schleiden did not realize what he had seen, Göttsche (1858) may be termed the real discoverer of mycothallism. In old thalli of *Pellia epiphylla* and of *Preissia commutata* he found a branched system of threads going from cell to cell which he at first considered as an individual vascular system but later recognized as fungal. Earlier in the history of mycorrhizal study it was supposed that fungi are commonly associated with the *Jungermanniaceae* (Leafy liverworts) but absent from the *Marchantiaceae* (Thalloid liverworts). Such at least was the opinion of Němec (1899), who supposed that the *Marchantiaceae*, being starch producers, could not have endophytes; and Stahl (1900) seized upon this erroneous suggestion and wove it into his ingenious hypothesis of mycotrophism. But it was soon made clear that symbiotic fungi are constantly found in many of the Thalloid liverworts.

Four orders of Hepatics are recognized, of which two, the *Ricciales* and the *Anthocerotales* have received virtually no attention from our workers. It is not likely that the *Ricciales,* which are mostly aquatic, should have endophytes; and there is but one report for these two orders, made by an early worker named Milde in 1851. He found what he termed "Kugeln" or little barrel-shaped spore-like bodies in the thallus of *Anthoceros, Riccia,* and other frondose hepatics, and he found that these "Kugeln" were made of little "cells" united in strings and that they never left the thallus voluntarily: "neither am I able", he said, "to make any observation as to their significance".

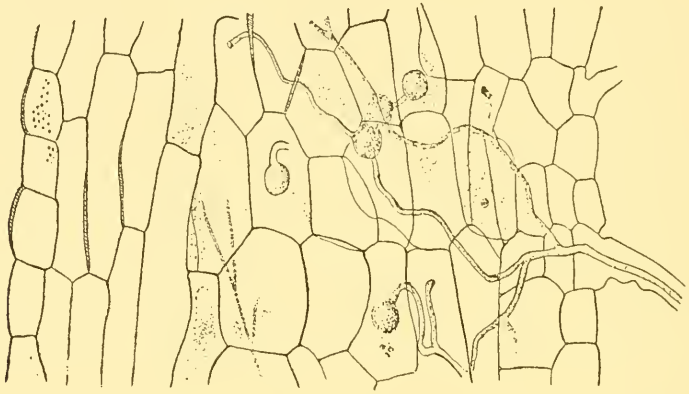


Fig. 1.—Longitudinal section through mycothallus of *Pellia epiphylla.* A fungal hypha, having entered through a rhizoid, ramified through tissues of the mycothallus and produced intracellular vesicles. (*Redrawn from* Ridler, Ann. Bot. 36:198, 1922).

Most of the work on mycothalli has been done on the *Marchantiaceae* and *Jungermanniaceae,* the Favourite Four species for study being *Conocephalus* (*Fegatella*) *conicus, Marchantia polymorpha, Lunularia cruciata,* and *Pellia epiphylla.* A greenhouse in Holland, a mountain area in India, a region in South Africa, the botanical garden of Java, and a few other spots all in Europe except one in Morocco—and none at all in the Americas—give us the rest of our information regarding the mycothalli of liverworts. Apart from the four species mentioned, only 37 other species have been reported on for their mycothallism and some of these reports are from the vague early days while others are mere casual mentions.

As to the other Bryophytes, we know virtually nothing of their possible mycothallism. The Sphagnums, being aquatic mosses for the

most part, would not be suspected of harbouring endophytes although it is true that a fungus has been reported from a *Sphagnum* capsule; the *Andreales* are not mentioned; and but one report comes to us of the higher mosses. SERVATTZ (1913) stated that white filaments of a fungus (the size of *Streptothrix*) formed dense intertangled masses which grew well on agaricized peptone bouillion, a fungus which seemed to be an *Oöspora.* This organism exerted a particularly activating action on *Phascum cuspidatum,* and in culture the moss plants and mycelium made normal growth when together whereas without the mycelium the moss developed only a protonema. This favourable action was negated later when the fungus covered over the gelose and gained ascendancy over the moss.

Fungal Symbiosis with Pteridophytes:—Fungal symbiosis seems to occur commonly with Pteridophytes and certain species, especially of the *Ophioglossaceae,* have been studied intensively. On the other hand, the families of *Matoniaceae, Hymenophyllaceae* and *Schizaeaceae* have never been examined for symbiotic fungi, so far as literature records; while many species in the remaining families are yet to be investigated. It is scarcely to be expected that the *Parkeriaceae, Marsiliaceae* and *Salviniaceae* should harbour mycorrhizal endophytes since these plants are aquatics; and we find that ASAI (1934) states that *Ceratopteris* and *Marsilea* are not mycorrhizal, thus confirming STAHL, who had also found *Pilularia globulifera* non-mycorrhizal.

Of all the Pteridophytes, the *Ophioglossaceae* have been most studied for symbionts. JANSE, who worked at Buitenzorg, had found branched hyphae and sporangioles in the third layer of cortex only, in *Ophioglossum pendulum;* while a few years later CAMPBELL (1907), working in the same place, found the same form of endophyte in both gametophyte and sporophyte, infection of the sporophyte occurring chiefly from the gametophyte. *O. moluccanum* and *O. simplex* have also been studied carefully and found to be characteristically mycorrhizal. *Helminthostachys,* at first reported to be without endophytes, was studied later (LANG, 1902) and found to be essentially similar in its symbiotic relationships to *Ophioglossum.* Fourteen species of *Botrychium* have been examined for endophytes and proved to be mycorrhizal: of these, twelve were studied by GREVILLIUS (1895) who stated that in these species hyphal formation always occurred in the roots. Both generations of *Botrychium* are mycorrhizal, infection taking place through the rhizoids.

All of the five genera of *Marattiaceae* have been studied and found mycorrhizal. The tree-like *Angiopteris* of the Orient tropics is re-

ported by several workers, the latest (STARK, 1925) finding the plant with endophyte in the Leningrad Gardens. In *Archangiopteris,* as in several others ferns, WEST (1917) found a new fungus that produced under natural conditions distinct reproductive bodies other than vesicles. *Marattia* itself, although reported non-mycorrhizal by STAHL, is attested by several later workers. CAMPBELL (1908) states positively the presence of endophyte in green prothallia of *M. Douglassi* besides those of several other *Marattiaceae,* including *Kaulfussia aesculifolia* which WEST (1917) confirms for the sporophyte plant. WEST also describes and figures infection for *Danaea alata* and *D. nodosa,* neatly demonstrating apparent phagocytosis within the outer layers of cortex.

The leptosporangiate ferns have been less studied but are not without their endophytes. Two genera of the *Osmundaceae* have been studied, VAN TIEGHEM as early as 1870 reporting mycelial hyphae of a parasite coiled about dark masses in large cells of the inner zone of cortex of *Osmunda regalis* and several other ferns. Strangely enough, STAHL asserts that this species is not mycorrhizal and no one else has made a later statement. CAMPBELL, in his studies of fern prothallia, found that many cells in *O. cinnamonea* and *O. Claytoniana* contain an endophyte which consists of large non-septate hyphae that are strictly intracellular. For the sporophyte of the last species, LOHMAN (1927) says that an endophyte is absent. In an excellent paper on *Todea barbara,* CRIBBS (1920) notes that an endophytic fungus was found to occur frequently in the cortical tissues of the root external to the endodermis and internal to the sclerenchymatous cells of the peripheral region. It was found to gain entrance by root hairs and also by dissolving its way through the epidermal cell-wall at the edge of the root-cap. CRIBBS gives us neat figures which beautifully delineate apparent digestion stages.

For the *Gleicheniaceae* our only author is CAMPBELL, who mentions five species of *Gleichenia* that have mycothallic prothallia. Two authorities sponsor the *Cyathaeaceae,* those Tree ferns of the tropics: JANSE found *Cyathaea* mycorrhizal in Java, pelotes and sporangioles being found in 3-4 layers of cortex; while ASAI (1934) reports *C. spinulosa* as mycorrhizal, and also *Alsophila formosana* and *A. pustulosa.*

The *Polypodiaceae* offer a hopeful although little touched field, for most of these ferns live in humus soil and might be expected to harbour endophytes; yet we suspect that lack of economic utility of ferns accounts for aversion to their study.

Amongst wildlings of the American prairies, LOHMAN (1927) gathered some casual specimens of fern and reported briefly as to

their being mycorrhizal or the reverse; and two species are thus reported by him for the first and only time, namely, *Cystopteris fragilis* (mycorrhizae occasional) and *Onoclea sensibilis* (ectotrophic); and he records *Adiantum pedatum* and *Pteridium aquilinum* also mycorrhizal. Doak (1927) finds the *Adiantum* species endotrophic while Asai (1934) reports on *A. flabellulatum*. *Pteridium* is undoubtedly mycorrhizal although the poll as it now stands is tied: Stahl (1900) and Takamatsu (1930) insisting that *P. aquilinum* is not mycorrhizal while Lohman (1927) and Rayner (1927) state that it is or appears to be, and Rayner clinches the matter by presenting a photomicrograph of the apparent endophytic fungus within the root tissues.

The large genus *Aspidium* is reported non-mycorrhizal by the four who have reported upon it,—Frank, Stahl, Hoeveler, and Lohman; yet surely these humus-dwellers deserve a reconsideration. So, too, with *Asplenium* which Stahl and Janse reported as without endophytes, and *Polypodium* which Stahl negated although Faber (1925) seems to indicate mycorrhizae for *P. Fêei*. Then *Nephrolepis* and *Blechnum* are recorded very casually only by Asai (1934) and deserve more study. Last of all in this brief list of studied *Polypodiaceae* is *Cheiropleuria*, which is of special interest because occurrence of endophytic fungi in prothallia of *C. bicuspis* var. *integrifolia* is added evidence in the author's opinion (Nakai, 1933) that the genus should be removed from the *Polypodiaceae* and be placed in a separate family, the *Cheiropleuriaceae*. This is the only case known to the writer where mycorrhizae are made of service as a taxonomic criterion.

Symbiotic Fungi among Arthrophytes:—There is almost no information extant regarding symbiotic fungi of the Equisetums. Sadebeck in 1875 described browning of the prothallia of *E. arvense* and *E. palustre* in culture, due to infection by a species of *Pythium* which was named *P. equiseti*, but this was apparently a case of parasitic attack rather than of mycothallism. Janse said that forest dwelling *Equisetae* in Java appear never to have endophytic fungi in the roots; Hoeveler (1892) found *E. hiemale* and *E. silvaticum* not mycorrhizal; and Stahl found no trace of infection in *Equisetum*. Lohman, in Iowa, lists *E. arvense* as containing an endophytic Phycomycete while *E. kansanum* was lacking in mycorrhizae. Detailed investigations of the Equisetums are yet to be made.

Mycorrhizae and Mycothalli of Lycopsida:—*Lycopodium* has received much attention. Following Treub's discovery of fungal infection of a Javan lycopod, Bruchmann (1885) described similar

infection for *L. annotinum,* the mycelium being both inter- and intracellular. Next, Goebel (1887) told how that in *L. inundatum* the lower non-meristematic part of the prothallium is always inhabited by a fungus. Janse followed with reports on 8 Javan species, several being reported for the first time. Holloway (1920) has added much to our knowledge of Australasian species, hence Europe and the Austral region have been partly covered but America has offered but two papers on lycopodiaceous fungal symbionts,—by Spessard (1922) and by Stokey and Starr (1924). Americans have produced six papers on mycorrhizae of ferns, two on Lycopods, and none on the hepatics.

Sixteen species of *Lycopodium* are reported mycorrhizal in the sporophyte while as to the gametophyte, the long-sought gametophytes of *Lycopodium,* discovered by Fankhauser in 1873 at Emmenthal, have likewise received attention and found to contain an endophyte. Whether this endophyte is a *Pythium* or an Ascomycetous fungus as Spessard claims, or of different sorts in different prothallia remains to be determined. The endophyte appears to be a mutualist.

Selaginella has received slight attention by students of symbiosis. Bruchmann (1897) found *S. spinulosa* mycorrhizal in the Alps while *S. helvetica* was not mycorrhizal. Janse said that *Selaginella* in Java possesses a fungus in the hairless roots. American species of *Selaginella* have never been reported upon for mycorrhizae.

The little family of the *Psilotaceae* which is segregated from the Lycopods and with similarities to the fossil *Sphenophyllineae,* has attracted a number of investigators most of whom agree that these plants are mycorrhizal; yet Costantin (1925, 1936) maintains it has been found without endophyte, not alone by himself but also by Noël Bernard. Solms-Laubach, Janse and Bernatsky were earlier students of the mycorrhizal condition, the last trying to isolate the fungus; while Shibata described cytological detail and called attention to phagocytosis occurring in the tissues and the similarity of the process to that occurring in the orchids. All of this work was done on the sporophyte but the gametophyte—a small colourless tuber embedded in humus—is likewise infected. A gametophyte supposed to be that of *Psilotum* was described by Lang but a detailed report was given by Darnell-Smith (1917). Sporelings of *Psilotum* are penetrated by an endophytic fungus after a comparatively few cell divisions and soon almost all of the cells of the prothallium are filled with a skein of hyphae, reports the author. Presence of the fungus does not cause a change in form of cell but the nucleus is frequently obliterated by its mycelium. Infection occurs near the growing point: hyphae are non-septate and two may occur at once in a rhizoid.

Hyphae have also been observed in antheridia and in canal cells of the archegonium but never in the egg-cell.

The work on *Psilotum* has presumably been done on *P. triquetrum* from which we may turn to the other genus of the family, *Tmesipteris*, a genus of Australian herbs. In the first of several papers on mycorrhizae, DANGEARD (1891) described endotrophic mycorrhizae from five species of *Tmesipteris*, telling of the fungi and their appearance in the root; and it would seem that he may have been dealing with both parasitic and mycorrhizal fungi. But he describes and figures Hartig net, hyphal coils, and notes disappearance of starch from the infected region. It is curious that the useful term, "mycorrhizome", should have been invented for the service of these plants so little known to the general botanical public; yet DANGEARD said that as roots are wanting in these plants, they may be said to possess mycorrhizomes. So came into being a designation for all endophytic creeping stems, especially amongst ferns and orchids.

The gametophyte of *Tmesipteris*, like that of *Psilotum*, contains an endophyte; for LAWSON (1917) in a paper complementary to that of DARNELL-SMITH described the infection in *T. tannensis*. Structurally the prothallium of *Tmesipteris* does not resemble that of *Lycopodium* but it does that of *Psilotum*.

Gymnospermous Mycorrhizae:—In all classes of Gymnosperms there are found mycorrhizal fungi occurring as endophytes. Among all the branches of the plant kingdom, none has attracted more research than that of the conifers; and especially have the pines been investigated. As early as 1865 NICOLAI had unwittingly described the mycorrhizal character of pine rootlets although it was not until 1873 that REINKE remarked the similarity of the cortical "thickenings" of the pine rootlets to those of the liverworts known to be due to fungi. Several reasons may be adduced for predilection for pine mycorrhizal research: first, modern mycorrhizal research began with the pines; second, the pines are easily studied; third, they are of great economic importance. About one-fifth of all mycorrhizal research in the last decade has been done with Gymnosperms, and of these principally pine, spruce and fir.

Mycorrhizae of Cycads:—Tubercles of the Cycads appear to harbour both fungi and bacteria and are modified rootlets. For them the name of "consortium" has been proposed, a name first suggested by GRISEBACH according to REINKE (1871) who quaintly observed that the term is "sehr zutreffend". LIFE, who made an extended study of these "consortia", declared that "In reference to the symbiotic rela-

tions which exist between these various organisms it is difficult to speak with any certainty . . . the tubercles of Cycads may be said to have at least two functions, that of aerating and that of assisting nitrogen assimilation." But whatever their structure or function, they can be considered only as a very special case of mycorrhiza, and the same may be said for the "nodules" of *Encephalartos;* while those of *Macrozamia* are reported by McLuckie (1922) to be purely bacterial. Spratt (1915), whose work on Cycadean nodules comes nearest to being monographic, states that all Cycadean genera produce root-nodules which are perennial modified lateral roots, repeatedly branched and forming large coralloid masses. They are primarily produced, he says, by infection with *Bacillus radicola*; and he asserts that the *Cycadeaceae* are the only nodule-bearing plants known in which four organisms are associated together symbiotically, *viz.* two nitrogen-fixing bacteria, an alga, and the cycad.

Mycorrhizae of Ginkgo:—Only one living member of this genus occurs and this member, the Maidenhair tree, has long since ceased to exist in a wild state. It is thus in the nature of an exotic wherever it grows, and its rooting conditions and structures are in a sense anomalous. Perhaps no other plant, the lone representative of its order, presents such an unique case; yet *Ginkgo* is reported mycorrhizal. Its earliest observer was Reinke (1873) who noted "thickening strips" in its root cortex; its latest observers were Klečka and Vukolov (1935) who state that the mycorrhizae are racemose, slightly furcate. Yet Schwarz (1883) and von Tubeuf (1896) reported abundant root-hairs for this species. An ecological study of *Ginkgo* roots in the native haunts of the species, so far as China could provide "native haunts", would be desirable.

Mycorrhizae amongst the Taxaceae:—The curious tubercles and necklace rootlets of various Podocarpi have proved fascinating to students of root structure. There is something which arouses curiosity in them: the roots are excavated, some lumpy excrescences appear, and forthwith the botanist hurries to his laboratory to see what meaneth this strange thing! A mere ordinary rootlet is passed by as commonplace: for example, the possible mycorrhizae of *Torreya* are virtually unknown, apparently because there is nothing about them to attract curiosity. But thanks to studies of the curious we have much on record about the nodules of ten species of *Podocarpus.* It appears that these nodules are called forth by bacterial action as well as by fungal invasion; but the consensus of opinion seems to be that they are often true mycorrhizae, being developed usually by a symbiotic

fungus although in cases a fungus is lacking. REINKE (1873), who saw much and described well, noted "thickening strips" in *Podocarpus* cortex; BERGGREN (1887), with the meticulous exactness of a Scandinavian, described in detail the pearl-necklace rootlets of the *Podocarpineae*, seeing in them a similarity in function to the velamen-covered roots of orchids. The similarity cannot be drawn too closely, however, for HILTNER (1899), in conjunction with NOBBE, demonstrated fixation of atmospheric N by roots of *Podocarpus,* which he considered as true endotrophic mycorrhizae; yet HILTNER suggests that Heaths and Orchids likewise may fix nitrogen. MCLUCKIE (1923) also found the *Podocarpineae* active in N fixation, stating that the process was accomplished by bacteria present in the cortical cells. On the other hand, SAXTON (1930) was unable to find bacteria in Tasmanian material of *Podocarpus*: "No trace of bacteria could be found but unmistakable and well-preserved mycorrhizal filaments."

HILTNER had considered these nodules as unformed roots but MCLUCKIE (1923) says that the nodules are modified lateral roots and arise from the pericycle, their normal growth being checked before they emerge from the cortex of the main root. Root-hairs, he says, are commonly present as VON TUBEUF had already stated. Yet it is necessary to be careful about accepting reports of root-hairs on mycorrhizal roots too readily, for setae of the fungus often simulate root-hairs; and MCLUCKIE himself says that the surface of the nodule and the main root is frequently invested with a loose tangle of fungal hyphae.

It is interesting to note that the term "prosporidi" of PETRI (1903) was originated from a study of species of *Podocarpus* growing at Florence in Italy. These spore-like bodies produced by the fungus, the sporangioles of JANSE, he called "prosporidi" on account "al loro significato morphologico più probabile". SHIBATA (1902) described in some detail the fungal structure and reaction, and reputedly demonstrated an enzyme in the mycorrhiza.

In addition to the nodules of *Podocarpus* are the mamillate or pearl-necklace rootlets described so well by JANSE (1897). In *P. cupressus* he found intermittent growth: "En général, après une courte interruption, la croissance reprend pour s'arrêter encore une fois dès qu'il s'est formé en second mamelon sphérique au sommet du premier. Cette croissance intermittente peut se répéter ainsi plusieurs fois de suite, mais au plus tard après le développement du cinquième mamelon l'arrêt est définitif." JANSE continues with a detailed description of the histological structure and origin of these mamelons or pearl-necklace mycorrhizae which are so widely found amongst conifers, casuarinas, *Liquidambar, Acer, Celtis,* and others.

The genus *Cephalotaxus* is virtually uninvestigated, for aside from notes by Reinke and von Tubeuf, there is only an observation by Prat (1926) that plants of this genus were abundantly mycorrhizal in the Arboretum at Angiers in France. *Torreya* has exactly the same record; and there is nothing whatever on record of the American *Torreya* which lingers in the Appalachicola hills of Florida. *Taxus,* being of more familiar presence, is better known as to its mycorrhizae: the older generation of mycorrhizal students noted it and in more recent days several have described it, particularly Prat (1926, 1934), who has made rather thorough studies of, first, the European *T. baccata,* and, second, the Canadian *T. canadensis.* The mycorrhizae in *Taxus* appear to be endotrophic mamelons or pearl-necklace beaded rootlets, and phagocytosis occurs in them. In his later paper Prat concludes that there is not a true mutualism but that the tree is a parasite on its parasite! Klečka and Vukolov (1935) record for *T. baccata* endotrophic mycorrhizae comparable to those of *Ginkgo.*

The genus *Pherosphaera,* sometimes doubtfully included in the *Taxaceae,* was studied by Saxton (1930), who found both species provided with nodules, but the Tasmanian species produced nodules more freely.

Mycorrhizae in Pinaceae:—Pines, orchids and heaths,—these are the mycorrhizal plants par excellence! Frank brought mycorrhizal study to the fore by his studies on pines and much of recent research has been concerned with these important economic trees. The first genus in the family for our consideration is *Juniperus,* the common juniper which, like the Yew, is of familiar presence. Its mycorrhizae are endotrophic (vide Klečka and Vukolov, 1935) and necklace-beaded but as Janse (1897) observed: "Les mamelons sont encore plus allongés et plus rares que chez le Cupressus. Au demeurant ils leur ressemblent beaucoup". Sarauw (1903) observed that in this species (the common juniper) an endotrophic mycorrhiza exists in conjunction with an Hartig net, which he says is the only case of the sort known, except that in *Cedrus Deodara* there is an Hartig net without a mantle. In recent days the mycorrhizae of *J. communis* have been monographed by Lihnell (1939) in an extended and well-illustrated paper. The American species of *Juniperus* are very little studied: McDougall and Jacobs (1927) state that *J. monosperma* is endotrophically mycorrhizal in the Central Rocky Mountains; Henry (1936) states that no mycorrhizae occur in *J. sibirica* and in *J. utahensis. J. sibirica* Burgsd. is the same as *J. communis* L. var. *montana* Ait., and *J. communis* is well known to be mycorrhizal in Europe.

The genus *Cupressus* has attracted no modern investigator except that Birch (1937) says that in New Zealand the fungus *Rhizopogon rubescens* appears to be a mycorrhizal symbiont of *C. macrocarpa.* For the Italian cypress, *C. sempervirens,* mycorrhizae were described by Janse and by Kirchner (1908), the former describing necklace-beads and the latter simple coralloid mycorrhizae with endotrophic mycelium. Berggren (1887) had stated that Hartig net is lacking;

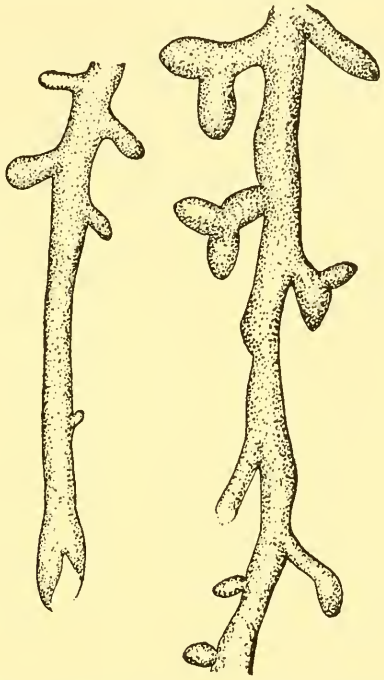


Fig. 2.—Mycorrhizae in *Pinus virginiana.* A "long-root" is beset with mycorrhizal short-roots or mycorrhizae, which in the older portion exhibit beginning of coral branching by dichotomy. The mycorrhizal sheath or mycoclene over the apex has split by renewed growth.

Yeates (1924) that the fungus is similar to that in Taxads. One other species, *C. Lindleyi,* was reported to have no root hairs by Schwarz (1883).

Chamaecyparis is even less studied than *Cupressus,* having no modern investigator except that Klečka and Vukolov (1935) list *C. Lawsoniana* as having endotrophic mycorrhizae. Noellê (1910)

reports for *Thuopsis dolobrata* and for the Incense cedar, *Librocedrus,* which last Yeates (1924) says contains a fungus similar to that in taxads. *Thuja* stands in a better position, being a more abundant tree in the cool temperate zone where most mycorrhizal students have lived. *T. occidentalis* and *T. orientalis* are both well studied while *T. plicata* and *T. Standishii* are reported as mycorrhizal. Two recent papers have cited the genus,—Klečka & Vukolov (1935) and Dominik (1937). *Taxodium distichum* has been listed as having endotrophic mycorrhizae but *Sequoia*—more attractive to curiosity—has been more studied. Both species of *Sequoia* possess endotrophic mycorrhizae, it would appear, and according to Strasburger, root-hairs are entirely wanting. Oddly enough, it is only European material of *Sequoia* that has been investigated while Californians neglect their most famous tree. *Cryptomeria* is reported mycorrhizal in Europe: von Tubeuf found root-hairs wanting in *C. japonica* although he notes that Klebs found sparse hairs on seedlings, which hairs were sloughed off with the outer cell layer. Mimura (1933), working at Tokyo, states that mycorrhizae are wanting on this species when planted at the Experiment Station but were found on roots that had grown from the pots into the ground. *Cunninghamia,* the China fir, is reported mycorrhizal by Noellê (1910) and by Yeates (1924); *Sciadopitys,* the monotypic Umbrella pine, by Noellê and by Laing (1923), the last describing the histology in some detail.

Four species of *Araucaria* are termed mycorrhizal. Janse compared its rootlets to those of *Podocarpus* but thought they were rather larger. Of modern writers we may note Rayner (1938) who in a review states that *A. Cunninghamii* grew in Nyassaland without inoculation of the soil; Young (1938) found that lime-induced chlorosis in this Hoop-pine was eliminated from some Queensland nursery beds by sulphur applications, and the same author found by pure culture experiments that its seedlings produced endotrophic mycorrhizae when grown in association with the fungus *Boletus elegans* and failed to develop in the absence of a mycorrhizal fungus.

The genus *Abies* has not proved attractive to our students although it appears to be mycorrhizal and material is abundant. Fifteen species of the genus are cited as mycorrhizal but without detailed description and with no details of physiological relationship. Of recent workers we may cite Dominik (1937) who notes three exotic species mycorrhizal in Poland; Colla (1931) who found three Basidiomycetes on *A. alba* in Italy; Tazoye (1940) who cites *A. Mayriana* as mycorrhizal in Japan; Henry (1936) who says that dwarfed *A. lasiocarpa* in the mountains of Utah is an excellent mycorrhizal host (Mc-

Dougall, 1927, had cited the same species from Idaho); and Klečka and Vukolov (1935) who list *A. alba* as ectotrophic.

Pseudotsuga is not popular with mycorrhizal students: but one of the four species has been investigated at all for mycorrhizae and this species only in a cursory way. In its native haunts it was described as both ecto- and endo-trophic by McDougall (1927) in Utah, and as having an endophyte in Canada by Lewis (1924); while Laing (1923) insists that this species does not form mycorrhizae readily. Klečka (1935) and Dominik (1937) find the species mycorrhizal in Europe while Birch (1937) records its fungi in New Zealand. *Tsuga* has received passing attention: five species are noted as mycorrhizal, of which one species, *T. heterophylla* is said by Laing to have semi-ectotrophic mycorrhizae the hyphae being found only between the cortical cells, and there is no mantle.

Twelve of the 39 species of *Picea* are cited as mycorrhizal and one of them, *P. Abies,* the Norway spruce, has been studied in detail by several investigators, especially Melin (1925). Using Melin's culture methods, Modess (1939) synthesized various Hymenomycetes with seedlings of *P. Abies*, a study further reported in 1941 when he listed 8 species of Hymenomycetes and one Gasteromycete that formed mycorrhizae with this spruce. Melin's methods were likewise used by Fries (1942) in synthesizing monospore mycelia of *Scleroderma aurantium* with spruce whereby mycorrhizae were formed but not as abundantly as with pine. Also in Sweden, Lindquist (1939) did his work on spruce and wrote philosophically on the physiology of mycotrophism; and Romell (1938) reports on his trenching experiments with spruce, and their bearing on the problems of mycotrophy. In another cultural study, Björkman (1940) reports on the ecology of the mycorrhizae of this spruce, while Thomas (1941) presents a plot study of young spruce plantations in the Rhine Valley. Besides the Norway spruce studied in Europe, various other spruces have been studied or noted in America and Japan.

Larix shares with *Picea* the attention accorded by Melin. Five species of *Larix* are recorded as mycorrhizal by various authors; and more recently How (1942) has made a monographic study of the mycorrhizal relations of *L. decidua.* Colla (1931) records *Hypholoma fasciculare* with the same species of larch; and Thomas (1941) notes larch plantations in the Rhine Valley. *Pseudolarix* is recorded only by Noell (1910).

Finally, in the Coniferales, we come to *Pinus,* the most studied genus of Gymnosperms. Thirty-seven species and varieties of *Pinus* are recorded mycorrhizal but of these only four have been studied in detail, namely, *Strobus, sylvestris, pinaster* and *montana.*

Mycorrhizae in Gnetaceae:—There are but two references to the possible mycorrhizal condition of these plants: (1) STRASBURGER said that root hairs are exceptional in *Ephedra,* while (2) KIRCHNER remarked that root fungus was not observed by VON TUBEUF and that root-hairs are not exceptional but found covering the roots for a distance of 2-3 mm.

The Method of Opportunism:—To summarize what is known of the mycorrhizae of Gymnosperms, therefore, one must say that much is known of a few pines and spruce and larch but that there is no general research upon the occurrence of mycorrhizae in the class as a whole. The same method of opportunism rules with the Angiosperms: there have been few scientific approaches to the mycorrhizae of higher plants through a systematic investigation of their occurrence. A few papers such as those of JANSE and of SCHWARZ point the way to a more thoroughgoing study of the rooting structures of Angiosperms; and meanwhile one pieces together the isolated papers to form the following picture.

Mycorrhizae in Apetalae:—First as to that collection chiefly of trees which has been called the Apetalae one notes that many are recorded mycorrhizal; indeed, the oaks and beeches are, with the pines, much studied plants. Of the poplars and aspens, *Populus,* seven species have been studied although not in much detail but their mycorrhizal character is established: KLEČKA and VUKOLOV (1935) are their only modern students. Sixteen species of *Salix* are given a similar character by various reporters, KLEČKA and ASAI being the most recent. The *Garryaceae* are unreported, for Mexico and the West Coast are almost untouched mycorrhizal fields. But the *Myricaceae* are much investigated because of their root-nodules which are true consortia (or mycodomatia), being occupied by bacteria and fungi simultaneously; and they are present in all members of the genus that have been studied—which are five of the 35 listed for the genus. Most of the work on *Myrica* has been done in Europe and Asia, almost none in America; but the American *Comptonia* is listed, by KELLERMAN. *Leitneria,* monotypic genus of the Corkwood family is unreported, and so, too, are the Asiatic *Platycarya* and *Pterocarya;* but the walnuts (*Juglans*) are recorded mycorrhizal. It is to be observed that FRANK and STAHL both stated that *J. regia* is not mycorrhizal, KLEČKA (1935) calls it ectotrophic, while the few reports on the two American species term these latter endotrophic. No detailed study of the walnuts is in print, nor of the hickories (*Carya*) except for that of the pecan (*C. pecan*) by WOODROOF

(1933). It is curious that trees so abundant as the hickories and so comparatively important should have escaped attention. Likewise the Betulas, for birches flourish in countries where mycorrhizal workers live; yet there is not much to report on them. The two more recent studies, of LAITIKARI (1934) and of BJÖRKMAN (1941) are concerned with the root system in general and with the concomitant fungi.

But with alder the case is different, for alder has "root excrescenses" that attract curiosity; and two European species attracted much attention in earlier days, KLEČKA (1935), PLOTHO (1941) and CERNIK (1937) being their modern students. HARSHBERGER was the only student of American alder while brief citations have come for Japanese species. PLOTHO (1941) tried the synthesis of alder nodules, which appears to be the only experimentation of the sort on record. Two species of *Carpinus* are reported mycorrhizal, the American species of *Ostrya,* and three species of *Corylus.* Coming, then, to the *Fagaceae* we meet with beech which, for some reason, has ever been popular at least in Europe, the latest study being by HARLEY (1939). It was on beech that MEYEN (1829) observed his "pseudomorphose" of the roots which may have been an unwitting discovery of mycorrhizae. Of chestnut, mycorrhizae were described on the European species by earlier students who thought to find in them a cause of disease of that economic tree. Chestnut provided KELLEY (1940) with his material for discovering the essential similarity between blight and mycorrhizal infection. But the Californian *Castanopsis* and *Lithocarpus,* with many species in Asia, are yet untouched. The oaks (*Quercus*) include "more than 200 species" of which 23 have been noted as mycorrhizal, one species only (*Q. robur*) having received some careful attention. Since oaks are preëminently American they offer a splendid opportunity for study of a vital function in important timber trees, especially open to those who say they have the interest of forests at heart,—a field of research that is virtually untouched.

Elm (*Ulmus*) was noted by one of our earliest students, DUHAMEL (1758), and since his time 5 species have been listed but no detailed study of any member of this genus exists. A couple of reports of "fungus-free" may be covered by STAHL's statement: "Wenn auch feineren Ulmenwurzeln des Oefteren sich pilzfrei erweisen, so trifft man doch hie und da innere Verpilzung." MCDOUGALL (1928) and JANSE (1897b) say that *Celtis* is mycorrhizal, and ASAI reports the same for *Zelkova* (*Abelicea hirta*) but otherwise nothing more is known of the mycorrhizae of the *Ulmaceae.* Similarly, 3 species of *Morus* are reputed mycorrhizal, being, like *Ulmus,* endotrophic; but we are as innocent of exact knowledge of

the mulberries as of the elms. Several *Ficus* have been termed mycorrhizal.

As to the herbaceous members of the Apetalae: Asai says that *Boehmeria* is not infected; Peyronel states that *Urtica* is: we can say no more for the *Urticaceae*. *Asarum* appears to be mycorrhizal in Europe, America and Japan by a single report in each case; *Rumex* by two reports; *Polygonum* by several. *P. viviparum* was said by Stahl to have "innere Verpilzung" while Asai and Takamatsu both state that certain Japanese species lack mycorrhizae. *Phytolacca decandra* also does not have any according to Asai. Two species of *Atriplex* are said to be mycorrhizal, the woody species were not in-

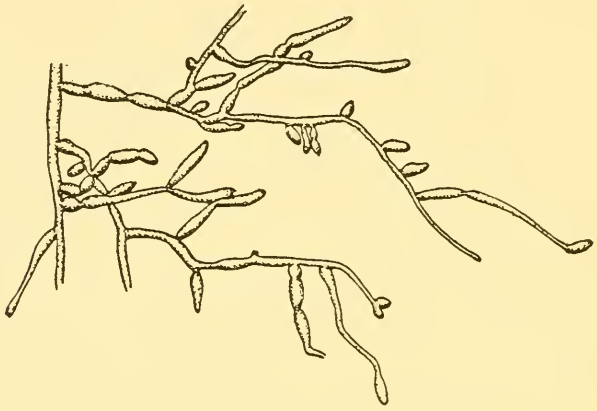


Fig. 3.—Mycorrhizae in Sugar Maple, *Acer saccharum*. A racemose system of mycorrhizae which are "beaded", due to periods of quiescence and renewal of growth. This mode of growth is characteristic of *Acer, Ilex* and other genera.

vestigated; *Beta vulgaris,* the common beet, is mycorrhizal but not *Salsola, Amaranthus sylvestris* was termed mycorrhizal by Trotter and *Scleranthus annuus* by Schlicht and by Stahl, while *Chenopodium* is mycorrhizal (Schlicht) or not mycorrhizal (Asai). *Portulaca* is positive while of the *Caryophyllaceae Gypsophila, Arenaria, Stellaria,* and *Cerastium* are in the plus column while *Dianthus, Silene* and *Sagina* are negative. This is the record of the herbaceous Apetalae.

Amongst the numerous Apopetalae and Gamopetalae there is a similar scantiness of information, the larger families showing a number of genera that have been examined casually for mycor-

rhizae while smaller families are entirely ignored. Thus, one dozen genera of *Ranunculaceae* are reported mycorrhizal and twelve of *Cruciferae;* twenty-six genera of *Papilionaceae* are reported which is the record for the Dicotyls; but as many or perhaps most of these cases are bacterial they are dubiously considered as mycorrhizal. The next largest record is of the *Compositae* with 25 genera reported.

There are conflicting reports: Thus, *Podophyllum* is termed mycorrhizal by LOHMAN and non-mycorrhizal by MACDOUGAL; *Benzoin* is mycorrhizal according to HENRY but non-mycorrhizal according to MACDOUGAL; *Ailanthus* is non-mycorrhizal according to STAHL and to DUTHIE but endotrophic according to KLEČKA and VUKOLOV.

Then there are families that are listed on the basis of single reports, as the *Calycanthaceae* (ASAI), *Menispermaceae* (MCDOUGALL & LIEBTAG), *Sarraceniaceae* (MACDOUGAL), *Pittosporaceae* (ASAI), *Sterculiaceae* (ASAI), *Tamaricaceae* (STAHL), *Cistaceae* (PIROTTA), *Nyssaceae* (HENRY), *Diapensiaceae* (ASAI), *Myrsinaceae* (ASAI), *Plumbaginaceae* (no mycorrhizae according to COSTANTIN); and these reports are not confirmed nor amplified.

In a number of families there is an amazingly large number of genera yet to be examined: Thus, in the *Borraginaceae* with 85 genera and 1500 species but 4 genera and 6 species have been studied for mycorrhizae; in the *Labiatae* but 10 genera and 14 species have been studied amongst the total of 160 genera and 3000 species; in the *Scrophulariaceae* but 6 genera and 18 species have been studied amongst 180 genera and 3000 species; while the *Bignonaceae* with 100 genera and 600 species is entirely untouched. In the great family of *Compositae* with its thousands of species there are but 54 studied for mycorrhizae.

In many of the Dicotyls we would expect to find phycomycete mycorrhizae as in the *Violaceae* of which 12 species are termed mycorrhizal but we have no detailed studies upon them. Two species of *Linum,* 3 of *Oxalis,* 3 of *Hypericum,* 7 of *Epilobium,* five of *Primula,* six of *Campanula* are listed as mycorrhizal; and we await further information as to whether the concomitant fungi are phyco- or basidiomycetes.

Several special cases may be noted: Thus the insectivorous plants have received some attention, the *Droseras* at the hands of FRANK, HOEVELER, SCHLICHT, and PEYRONEL; *Sarracenia,* of MACDOUGAL. STAHL was interested in the *Polygalas,* terming them

endotrophic. Four species of *Euphorbia* are cited but *Asclepias* which would seem to be of equal interest as a lactiferous plant has escaped observation except for two reports on *A. syriaca.* D'Angremond and Hell (1939) describe endotrophic mycorrhizae for *Hevea.* Three species of *Cactaceae* are cited, by Johansen (1931) except that Asai also cites *Neomammillaria. Monotropa* was long a focal point of interest. Twenty-one species of *Gentiana* are cited, and *Obolaria* is noted.

The woody Dicotyls have fared better, and we may run briefly through the list: The *Magnolias* and *Liriodendron,* and the tropical *Talauma, Manglietia* and *Michelia* (according to Janse); *Merati* of the *Calycanthaceae* (Asai); *Asimina* of the *Anonaceae; Sassafras, Benzoin* and *Ocotea* of the *Lauraceae; Pittosporum* (Asai); *Liquidambar, Altingia* and *Hamamelis; Platanus;* 9 genera of the *Malaceae,* four of the *Rosaceae,* nine species of *Prunus;* two of the *Mimosaceae,* 3 of the *Caesalpinaceae* and 7 of the *Leguminosae;* four of the *Rutaceae* (including 3 spp. of *Citrus*); *Picrasma* and *Ailanthus; Melia* and *Dysoxylum,* two species of *Buxus;* 3 of the *Anacardiaceae,* 3 species of *Ilex,* 2 genera of *Celastraceae;* 3 of *Staphyleaceae;* 8 species of *Acer; 7* of *Aesculus; 7* genera of *Sapindaceae;* 4 of *Rhamnaceae;* 2 of *Vitaceae;* 5 species of *Tilia; Firmiana* of the *Sterculiaceae; Thea; Tamarix;* 8 species of *Daphne,* 3 genera of *Elaeagnaceae; Nyssa sylvatica* (Henry); 3 spp. of *Eucalyptus;* 3 genera of *Araliaceae,* 2 of *Cornaceae; Clethra* (Asai); 21 genera of *Ericaceae;* including 13 spp. of *Vaccinium; Diapensia* (Asai); *Ardisia* (Asai); *Diospyros* (Asai); *Symplocos* (one species out of the 290 spp. in this monotypic family!); *Styrax* (Asai); 5 genera of *Oleaceae;* 2 species of *Nerium; Gardenia* (Asai), 4 genera of *Caprifoliaceae.*

For more recent work we may note: Milanez (1940) records root fungi for *Citrus aurantifolia,* said to be the first record for South America, but he considers them as parasites; Muller (1936) reports on mycorrhizae of citrus in the Netherlands Indies; Reed & Frémont (1935) and Rayner (1933) describe a phycomycete mycorrhiza for *Citrus* and regard it as beneficial under certain conditions. Berkeley (1936) states that raspberry roots (in Canada) show a phycomycetous infestation similar to that in strawberry, as recorded by Richards & McKay (1936). Bouwens (1937) considered the strawberry endophyte to be a *Rhizoctonia,* which generic fungus was also responsible for mycorrhizae in quince (*Cydonia*). A phycomycete mycorrhiza is described likewise for almond (*Amyg-*

dalus) by Ruggieri (1937). In Cacao in Trinidad, mycorrhizae also occur (Pyke, 1935; Laycock, 1945), although not invariably. Sabet (1939) describes mycorrhizae for cotton (*Gossypium*), and Tunstall (1940) for *Thea.*

For other plants, Heath and Luckwill (1938) report mycorrhizae in *Potentilla* and several other heather-land plants; while Malan (1938) studied mycorrhizae of alpine legumes (finding them phycomycetous). For *Ericaceae,* Barrows (1936, 1941) studied *Epigaea,* and Freisleben (1933, 1934) particularly *Vaccinium*; Gordon (1937), *Rhododendron;* Bain (1937), after studying *Oxycoccus,* comes to the conclusion that there is no obligate symbiosis while Rayner & Levisohn (1940) contradict him; Molliard (1937) after studying *Calluna,* concludes that mycorrhizae are not essential. For potato (*Solanum*), Costantin (1935, 1936) and Joseph (1935) present data. Kürbis (1937) and Kelley (1943) have described mycorrhizae for *Fraxinus;* Schimmler (1937) for 12 spp. of *Gentiana.*

Since Monocotyls are not woody, less interest can be expected in them. It is true that there are some monocotyledonous trees which are reported mycorrhizal,—the palms *Phoenix* and *Livistona,* the screw-palm, *Pandanus,* and the banana "tree", *Musa.* But most Monocotyls are herbs, and many are aquatic plants in which no mycorrhizae are found, as *Typha* (Asai); *Alisma* (Asai); *Calla palustris; Acorus,* 2 spp.; and 10 spp. of *Juncus* which, however, produce root swellings that do contain a fungus according to Magnus. No mycorrhizae are reported for the *Cyperaceae; viz.* 2 spp. of *Cyperus,* 3 spp. of *Eriophorum,* and 14 spp. of *Carex;* but numerous species of grasses are reported mycorrhizal. For the *Gramineae,* Asai (1934) reported 23 species mycorrhizal and 4 not mycorrhizal, the latter all hygrophylls; while 58 spp. were reported mycorrhizal by other observers. The latest researches on grasses are by Biraghi (1936) on cereals, and Neill (1940) on *Lolium.* Of the aroids, *Arisaema* is mycorrhizal (Lohman, 1927), while Magrou (1937, 1939) used *Arum* for isolation of the endophyte.

The *Liliales* seem richly mycorrhizal: *Veratrum* in the *Melanthaceae, Allium, Lilium, Tulipa, Erythronium, Ornithogalum, Muscari, Hemerocallis, Yucca, Fritillaria, Scilla* and *Aloë* in the *Liliaceae; Asparagus, Smilicina, Maianthemum, Uvularia, Polygonatum,* and *Convallaria* in the *Convallariaceae;* and *Trillium* in the *Trilliaceae.* Oddly, there is no report for *Smilax.* Then *Narcissus, Galanthus, Leucojum* (Stahl) and *Agave* (2 spp.) of the

Amaryllidaceae (but no mycorrhizae in *Aletris* [TAKAMATSU]); *Dioscorea* (ASAI); 4 genera of *Iridaceae; Zingiber* and *Musa* of the *Scitaminaceae; Ananas* of the *Bromelidaceae;* while the *Burmannias* have attracted much interest, the latest record by CIFERRI (1946). The orchids would require a separate section to do them justice, for no less than 85 genera are described as mycorrhizal while 20 papers on orchid mycorrhizae have appeared in the last decade.

Lecture III

THE FUNGAL ENDOPHYTES

Nature of the Mycorrhizal Fungi:—It scarcely needs to be said that mycorrhizal fungi are not a separate taxonomic unit in the classification of fungi. They are the ordinary soil fungi of forest and woodland, of meadow and cultivated field. Neither are they special members amongst the congeries of soil fungi in the sense that one, and only one, member can achieve a mycorrhiza. One fungus or another can produce it, and ordinarily there may be several fungi participating, forming what has been called a "multiple mycorrhiza". In other words, the fungi living in the soil grow into plant roots as into a part of their environment, and, if the host plant is able to check the fungus in its rootlet cortex and break down the fungal hyphae, the association is said to be mycorrhizal. Presence of the fungus, regardless of its taxonomic identity, has little to do with the form of the mycorrhiza, which is characteristic for a given host plant and is determined by the host. In an informing paper by MAGROU, DOUCHEZ & SEGRETAIN (1943), it is shown that mycorrhizae are formed with potato by various endophytes some normally present with monocotyledonous, some with dicotyledonous plants. The endophytes present in various soils simply grew into the potato roots and gave the stimulus to the production of characteristic tubers. It was the potato plant that determined tuber form, not the fungus.

The mycorrhizal association, therefore, appears more as a casual thing than as an occult and premeditated action that can be achieved only by special, designated actors. It is true that certain fungi do seem more or less confined to certain mycorrhizal hosts, although specificity cannot be said to be absolutely proved; but a certain amount of specificity could be posited on the grounds of chemical affinities. The emphasis that has been placed on mycorrhizal fungi would seem, therefore, to be somewhat exaggerated because in so many cases the identity of the fungus seems a relatively inconsequential thing. It is nutrient that the higher plant requires and in many cases it seems of little moment whether the particular fungus which supplies the nutrient happens to be a *Russula* or an *Amanita,* a *Boletus* or a *Tricholoma.* These are the fungi of the forest floor and naturally have to be the mycorrhizal fungi of the trees that grow there. It would seem

logically deducible that the only fungi available to forest trees for formation of mycorrhizae would be those of the forest soil; while phycomycetes of cultivated ground are available to crop plants. Nevertheless, there is a physiological separation possible amongst soil fungi, according to MELIN (1925), who recognized three groups of these fungi, *viz.,* symbiophiles, saprophytes and parasites. All "mycorrhizal fungi" are considered as symbiophiles.

Some investigators, wishing to prove that sporophores of *Russula,* etc., which appear on the forest floor are actually part of the mycorrhizal mycelium, have laboriously traced that mycelium from the sporophore to the mycorrhiza and thereby established, so they said, the identity of that particular mycorrhizal fungus. But their success was denied by other investigators who asserted that attachment of a sporophore to a mycorrhiza is no proof whatever that the fungus concerned is mycorrhizal; for who can say but that this sporophoric fungus is not a secondary parasite? Therefore, say these later students, the only thing to do is to grow the fungi in pure culture, inoculate them into sterile seedlings, and if a mycorrhiza results there is positive proof of the identity of the mycorrhizal fungus. But is there positive proof? Laboratory experiments show what can happen in the laboratory but not what happens in nature. A laboratory synthesis of *Boletus granulatus* with pine shows by its production of a mycorrhiza that this fungus is capable of such production but it does not prove that mycorrhizae produced on pine in nature were produced by *B. granulatus.* They might have been produced by another fungus growing on the same area. When only a single fungal species has formed sporophores over the roots of pine and when that species is shown by synthesis-experiment to be able to produce mycorrhizae, then it can be said with justice that this species is the mycorrhizal fungus in question; but one could have come to that conclusion without experiment. Or, to use ROMELL's (1939) illustration: *Lactarius delicosus* has been grown on pine in the laboratory but in nature it rarely if ever is found on pine. In other words, the various lines of research used with reference to mycorrhizal fungi all help to identify the fungi; but the question of identity is after all not of major importance.

In earlier days of mycorrhizal research, it was thought that mycorrhizae were produced on trees by basidiomycetes and that herbs in general lack mycorrhizae; but with greater development of microscope and technique it is known that all major fungal groups furnish mycorrhizal fungi. We shall consider them in the usual systematic order.

Phycomycete Mycorrhizal Fungi:—The records for these fungi before 1920 are somewhat uncertain because it was not until recent years that phycomycetous mycorrhizae were regarded as constant features of nature. It is true that TREUB, BRUCHMANN, and GOEBEL had independently found *Pythium* in prothallia of lycopods; while JEFFREY had assigned the endophyte of *Botrichium* to the same genus. DANGEARD had found a chytridiaceous fungus on *Tmesipteris* which he regarded as mycorrhizal; and there are a few other records of the same sort.

It was PEYRONEL who brought the "Phycomycete mycorrhiza" to our attention, commencing in 1922 with a study of cereal grains that were brought to his station for a study of diseased condition. PEYRONEL found that these cereals, instead of being autotrophic, possessed mycorrhizal infection,—the infection being considered mycorrhizal because the hosts were "perfectly normal". From this study PEYRONEL continued: He saw quickly that endotrophic fungi are of two major sorts,—the first possessing arbucles and vesicles and the second only mycelial pelotons (found chiefly in orchids except that MOLLBERG found vesicles in certain orchids). Later (1924) PEYRONEL described three species of *Endogyne* involved in formation of endotrophic mycorrhizae on herbaceous phanerogams, the first characteristic of peaty, swampy soils, the second exclusively hydrophilous, and the third found on *Euphorbia dulcis*. Other species of *Endogyne* were reported in 1937 from the Val Valdesi, producing endotrophic mycorrhizae on *Viola* and other herbs. In the same year, he published on endotrophic mycorrhizae of the Alps at Kleinen St. Bernhard, and noted that conditions in a cultivated garden were markedly less favourable for growth of the mycorrhizal fungi than in the natural habitat.

Interest in the phycomycetous mycorrhizae had been stimulated by JONES (1924) in a publication in which he recorded the discovery "that the roots of nearly all our common leguminous crops, wherever grown, are extensively invaded by a characteristic fungus which has previously been known as a mycorrhizal fungus. So abundant is this fungus that it appears unlikely that many plants of alfalfa, clover, peas, and other legumes ever reach maturity without having their roots more or less invaded. . . The taxonomic position of the fungus has not been determined but it appears to belong among the Phycomycetes." JONES gave a list of other plants besides legumes in which this same sort of mycorrhizal invasion had been found.

This paper of JONES' inspired SAMUEL (1926) to work in South Australia, and he reported the same sort of infection in 27 legumes, 30 *Gramineae,* and in herbs of the families *Liliaceae, Ranunculaceae,*

etc. Other workers continued the reports, and, in 1935, Rayner remarked on "the remarkably widespread geographical distribution of this 'Phycomycete type' of mycorrhizal association, its prevalence in plant species of the most diverse affinities (and) its recorded appearance in certain crop plants." Biraghi (1936) confirmed Peyronel on the frequence of endophytic infection of roots of cereals, finding *Asterocystis radicis* in a majority of cases. Ruggieri (1937) reported endotrophic mycorrhizae common on fruit trees, a Phycomycete being constant in root cortex of almond. Berkeley (1936) found a phycomycetous mycorrhizal fungus on raspberry in Canada; Richards & McKay (1936), on strawberry in Utah; and Reed & Fremont (1935), on *Citrus*.

In 1939, Butler published a paper devoted to a study of "the distribution and morphological characters of the vesicular-arbuscular or Phycomycetoid endophytes which commonly occur in cultivated and probably other soils forming mycorrhizal associations in the roots of many flowering plants and cryptogams, including prothalli of liverworts and of some ferns. The regularity of their occurrence in some annual field crops is believed to be merely the result of the greater opportunity to persist indefinitely, by passing from the older to later developed roots, offered to the organism in perennial plants." Believing with Peyronel that these fungi belong to the *Endogynaceae,* Butler cites Dangeard's (1898) name of *Rhizophagus* for their genus, and describes the species as *R. populinus, R. theae* and *R. marratiaceum.* Sabet (1939) promptly placed on record the presence of *Rhizophagus* sp. as the mycorrhizal fungus of cotton in the Sudan.

The first reputed synthesis of a Phycomycete mycorrhiza is said to have been that of the unnamed endophyte of *Arum* with roots of *A. italicum* (Magrou, 1936).

Ascomycetous Mycorrhizal Fungi:—Various Ascomycetes have been cited in connection with mycorrhizae, as *Aspergillus* and *Penicillium* (Ternetz, 1907); *Terfezia* (Pirotta, 1900); *Mollisia* (Němec, 1899); and *Humaria* (Nicolas, 1929); but *Elaphomyces* and *Tuber* are the most frequently reported of the group. Very early, Boudier (1876) had noted presence of *Elaphomyces* on low ground with *Molinia,* a grass; or on higher grounds where *Leucobryum* moss was growing. Still earlier (1837), Berkeley had cited the association of *E. muricatus* with beech roots in mountainous woods. Tulasne (1841) remarked that *E. granulatus* is confined to roots of one sort of tree (not named) and "flourishes when tree is active." This same species Boudier had found on birch, oak and

chestnut at Nancy in France. Reess (1880) observed coralloid clusters of mycorrhizae on pine bound with mycelium of *Elaphomyces,* while Lewton-Brain (1901) described mycorrhizae of pine formed in conjunction with *E. variegatus.* Since then interest in *Elaphomyces* has languished.

The genus *Tuber* also attracted observers, *e.g.* Frank (1888) who observed *T. aestivum* on beech; and more latterly Costantin (1924) who found that ascospores can be formed by the fungi apart from mycorrhizal symbiosis. Mattirolo wrote a number of papers on truffles, finding (1934) the mycorrhizal fungus of the introduced *Populus canadensis* to be *T. Borchii;* and he suggested the possible introduction of fungus with the tree when the latter was brought to Italy from California.

Hemibasidiomycetes:—Of the *Hemibasidiomycetes* may be noted the following: Weber (1884) assigned the fungus responsible for tuber formation in *Juncus* to *Entorhiza* of the *Tilletiaceae.* Lagerheim (1888) described a new species of *Entorhiza* from roots of *Juncus articulatus* obtained in Switzerland. The fungus had caused the roots to form into galls, and within was an abundance of yellow "spores". In the Black Forest similar nodules were found on the same species of rush, and similarity to leguminous nodules was pointed out. Formation of nodules on several species of *Juncus* was noted by Schwartz in 1910.

Hymenomycetous Mycorrhizal Fungi:—These are the chief mycorrhizal fungi. Upwards of 50 genera of Hymenomycetes have been reported as forming mycorrhizae (or perhaps it should be said, incriminated in their formation); but in most of these cases there are only one or two species cited in one or two reports. The principal "mycorrhizal fungi", if numbers of reported species mean anything, are *Boletus, Amanita, Lactarius, Cortinarius, Russula,* and *Tricholoma.* Commencing with Frank's (1888) observations on *Boletus bovinus* with spruce, later observers—almost all since 1920—have shown by field observation and synthetic experiment the connection of about 30 species of boletes with various trees. In a few cases a bolete has failed to form mycorrhizae in synthesis, as *B. edulis* with pine and spruce (Modess, 1941); while *B. parasiticus* is a parasite as the name indicates (Smotlacha, 1911). Smotlacha believed that certain boletes are confined to the neighbourhood of certain trees, as *B. rufus* with aspen and *B. rugosus* with beech.

The *Agaricaceae* are much investigated mycorrhizal fungi, although *Agaricus* itself provides few members that are endophytes.

Amanita, so common in woodlands of Europe and eastern America, has been studied, commencing with Boyer's (1915) observation that the mycelia of many mushrooms, especially of Amanitas and boletes, extend to mycorrhizae of neighbouring trees. *A. muscaria* seems the principal mycorrhizal fungus of this genus, and was shown by Modess (1941) to form mycorrhizae with pine and spruce. No less than 17 species of *Lactarius* are said to be mycorrhiza-formers, and of these *L. delicosus* and *L. rufus* are the chief, being confirmed by synthetic experiment. All of these reports come from Europe, except for three citations by Hatch (1937) for American material. Americans have listed three species of *Clitocybe* as mycorrhiza-formers, but Modess, in synthesis experiment, reports none of the six species he investigated as forming mycorrhizae. A considerable number of species of *Cortinarius* are said to be mycorrhizal but detailed studies are lacking in almost all cases; and the same may be said for the 18 species of *Russula* that are alleged to form mycorrhizae. *Tricholoma* has fared better, especially at the hands of Melin and Modess; but the latter reports 4 species of the genus that failed to form synthetic mycorrhizae.

Some special cases among the Hymenomycetes may be cited. The polypore *Strobilomyces strobilaceus,* a widely distributed woodland species, was stated by Peyronel to be connected with *Corylus Avellana.* The Hydnums and most polypores one thinks of as bracket fungi on wood, but Masui (1927) states that *H. affine* "was determined" as a mycorrhiza-former with *Pinus densiflora; Polyucomelas* was mycorrhizal also on this pine; while Long (1913) stated that *Polyporus Berkeleyi* had been found on larch in Montana. the fungus securing food from the forest humus,—which may or may not have meant that the species was mycorrhizal. Amongst the agarics one would suppose that *Lepiota* would surely be a mycorrhiza-former, but Modess (1939) obtained uniformly negative tests in attempting synthesis with species of this genus. *Amanitopsis vaginata* is mycorrhizal in Europe according to Peyronel and Modess. The species is common also in America but is not reported mycorrhizal. On the other hand, *Cantharellus cibarius,* which also occurs both in Europe and America, is reported mycorrhizal only by American workers (Doak, 1934; Thomas, 1941.) *Hygrophorus,* having a viscid cap, includes *H. virgineus,* which is mycorrhizal on spruce (Frank, 1888), and *H. Bresadolae* and *H. lucorum,* on larch (Peyronel, 1922). *Omphalia,* which we think of as tiny fungi of damp leaf-mold, is mycorrhizal on *Nothofagus* in New Zealand; and the Fairy-ring fungus, *Marasmius oreades,* is mycorrhizal with *Pinus*

ponderosa (Birch, 1937). *Armillaria* is mycorrhizal only in Japan, so far as records go.

Whatever spore-colour may, or may not, have to do with it, the great majority of mycorrhiza-forming agarics are white-spored, *Cortinarius* being the only important exception.

Gasteromycetous Mycorrhizal Fungi:—The record for the Gasteromycetes is much shorter. For *Lycoperdon,* McArdle (1932) stated that *L. gemmatum* formed mycorrhizae in synthesis with *Pinus Strobus* and *Picea nigra,* and he implicates *L. pulcherrimum* also in mycorrhiza-formation. Birch (1937) found *L. perlatum* mycorrhizal on *P. laricio;* but Modess (1939, 1941) said that this fungus failed to enter into synthesis; also *L. pyriforme.* Similarly, McArdle regarded *Calvatia saccata* as mycorrhizal, but Modess says that this species did not enter into synthesis. Again, Noack (1889) implicates *Geaster fimbriatus* and *G. fornicatus,* but Modess says that *G. minimus* did not enter into synthesis; and Melin (1925) also failed to secure synthesis. *Scleroderma* has a better record since three species,—*aurantium, bovista,* and *vulgare,*—are fully attested as mycorrhizal, with even Modess (1941) agreeing on the first. In South Africa, *Polysaccum crassipes* is mycorrhizal on *Eucalyptus,* and shows phagocytosis unusually well (Smith & Pope, 1934).

Phallomycetous Mycorrhizal Fungi:—Only one record appears for the Phallomycetes, *viz.* that offered by Barsali (1922): Mycorrhizal-like mycelia on roots of *Robinia Pseudo-Acacia* were seen in fruit to be *Clathrus cancellatus;* and in the same way the fungus was found in gardens on roots of *Phyllostachys bambusoides* and *P. nigra.*

Form Genera of Mycorrhizal Fungi:—The "form genera" of mycorrhizal fungi have yet to be considered. These homeless waifs of mycological taxonomy have been adopted by ardent mycorrhizologists and given cognomens which do not relate them to any other fungi but do enable the student to talk about them conveniently. That is, convenience with some reservations, for, confronted with such scientific names as *Mycelium radicis Walycwi* or *Mycelium radicis Didymoplexis pallentia,* one wonders whether taxonomy may not have reverted to pre-Linnaean habits. Melin goes still further and speaks of *M.r. abietis, alpha, beta, gamma,* etc.

Fusarium:—The form genus *Fusarium,* established by Link in 1809, is the longest cited form-genus in connection with mycorrhizae.

As early as 1847, Reissek was isolating a fungus from the "root" of *Orchis Morio* which he assigned to this genus and named *F. endorrhizum;* while, in 1890, Vuillemin cites a *Fusarium* from *O. mascula;* in 1900, Bernard, from *Ophioglossum vulgatum.* In 1901, Bernard said that tuber-formation in the potato is called forth by an endophytic fungus, *F. solani.* The fungus is now generally distributed in European soil and potatoes form freely, but at first potatoes grown from seed did not form tubers until the soil was inoculated with fungus. The next year Bernard stated that the fungi concerned in all tuber-formation are *Fusarium* spp., conidial forms of which are near to the related genera of *Nectria* and *Hypomyces* except that the fungus of potato is *F. solani.* But, in 1904, Bernard decided that the Fusaria often obtained from orchids are not the specific fungi since they do not cause germination; and the endophyte, he decided, as obtained from *Cattlyea* is a fungus described by Bernard as *"Mucédinée oösporée."* The following year he said that, while the endophyte of *Cattlyea* has structures similar to those of *Oöspora,* that from *Odontoglossum grande* is similar to *Rhizoctonia;* and to *Rhizoctonia* Bernard adhered during the rest of his brief life.

Rhizoctonia:—The sterile fungus, *Rhizoctonia,* which in one case at least (Sprau, 1937) is identified with *Corticium,* has been much talked of since the days of Bernard; indeed, many botanists had the idea that study of mycorrhizae was largely the study of these fungi. Most of the fungi isolated from orchids in those days were identified as species of *Rhizoctonia,* for example: *R. languinosa* (Bernard, 1909), *R. Goodyerae repentis* (Costantin, 1920), etc. More recently other species have been cited, as *R. repens* (Knudson, 1925), *R. mucoroides* (Porter, 1942).

Phoma:—The genus *Phoma,* with conidiospores in pycnia instead of on conidiophores as in the Rhizoctonias, has been cited a number of times. Ternetz (1907) studied five species assigned to this genus, which she isolated from native German *Ericaceae;* while Rayner (1915) found a fungus in *Calluna* which she placed in a new genus, *Phyllophoma,* since it occurred not alone in the root but throughout the whole plant. From *Vaccinium Oxycoccos,* Addoms (1931) isolated *Phoma radicis.* But in his study of root fungi of *Vaccinium,* Freisleben (1934), who isolated the mycorrhizal fungi, said that they were apparently not to be referred to the genus *Phoma,* to which other authors had assigned the endophytes of the *Ericaceae.* As to other plants: P. R. White (1929) separated several fungi from mycorrhizae of *Fragaria* and thought that a *Phoma* was responsible

for the mycorrhizae. AURET (1930) found a *Phoma* sp. in *Lunularia* in South Africa; RIDLER (1922) in *Pellia* and (1923) in *Lunularia* in England, but was not certain in the latter case that *Phoma* was the true endophyte.

Mycelium Radicis:—The older names of *Fusarium* and *Rhizoctonia* were supplanted in 1909 by BURGEFF's new name of *Orcheomyces* which he applied to fifteen orchid fungi. The name of "Orcheomyces" is attractive: it is short and expressive, but apparently only NOBÉCOURT (1923) adopted it; and in 1911 BURGEFF had abandoned the name and adopted *Mycelium radicis* in its stead. This name is of more general application but it is awkward, even though abbreviated to *M. r.,* and it violates the Linnaean principal of binomialism. MELIN adopted the designation for his isolates, *M. r. abietis* from spruce and *M. r. silvestris* from pine. Most of these fungi are basidiomycetes but *M. r. atrovirens* is a phycomycete and a parasite that forms pseudomycorrhizae (MELIN, 1921). Associated with this fungus may be another distinguished by a mycelium of coarse, lustrous, jet-black hyphae that radiate from the mantle of a mycorrhiza, a fungus which was named *M. r. nigrostrigosum* by HATCH (1934). This fungus was apparently figured by GIBELLI (1898) and is described by MANGIN (1899). BJÖRKMAN (1941) found both these fungi in Sweden, under stands of spruce, pine and birch.

As the designation *Mycelium radicis* usually (but not always) refers to Basidiomycetes, so the recently prominent *Rhizophagus* refers to Phycomycetes. BUTLER's (1939) paper on this genus had already been referred to in an earlier paragraph.*

Conclusion:—In conclusion, we may say that there seems to be an unnecessary emphasis laid on the fungal endophyte. If it were shown that one fungus is more capable of proteolysis than another and therefore better able to invade tissues of a plant; or if another fungus had a greater supply of diastasic enzyme and was consequently better fitted to be an orchid symbiont; or if yet another fungus had rich provision of N cation or phosphorus-complex and was therefore a richer "booty" for the "mycorrhiza to capture", there would seem to be some point in the emphasis laid on fungal identification. But in all cases it is simply a case of: A occurs on B, or C occurs with D; when, as a matter of fact, we know that A and C—and E and G, for that matter—can all occur in the mycorrhiza of B at the same time.

*LIHNELL finds that *M.r. nigrostrigosum* is the same as *Cenoccoccum graniforme* (Symbol.bot.Upsaliens. 5(2), 1942).

Even though one were to say it is necessary to know fungal identity to distinguish between beneficial and parasitic species, the argument breaks down before the realization that, strictly speaking, there are no mycorrhizal fungi: there is only a mycorrhizal state. Apparently almost any fungus can be a non-pathogenic symbiont; but nature of the symbiosis depends on a complex of physiological and ecological conditions or influences, and not necessarily upon any specific fungus, and the non-pathogen under different circumstances may become a pathogen, or the reverse. Since there is apparently no specificity in mycorrhizal endophytism, and since no analyses of mycelia are made to determine specific differences, the identifying of the mycorrhizal endophytes must be regarded somewhat in the nature of an hobby. It is important, just as every scientific discovery is important, but its importance would seem to consist chiefly in allaying our curiosity as to what fungi can enter into mycorrhizal symbiosis. It is something like discovery of mountains in the Antarctic,—very interesting but of no obvious utility.

Lecture IV

FOSSIL MYCORRHIZAE

Limitations of the Fossil Record:—Since 1904, when the first paper on fossil mycorrhizae appeared, enough information has been gathered to outline the fossil record of our subject. Yet this record has grave limitations, imposed not alone by scantiness of the investigations but by the nature of all palaeobotany. We have become so accustomed to thinking in the terms of Historical Geology that ofttimes we forget the "geological time table" was created a century ago, when knowledge was far more deficient than it is today, and that later discoveries have been pieced into the Lyellian system, the resultant table being far from convincing. It is of interest to observe that Historical Geology is one of the few sciences, perhaps the only science, that has not undergone major revision in the current century; and, whereas Newtonian Physics has been supplanted by Einsteinian Physics and other sciences have been critically reworked, Historical Geology continues unrevised. Indeed, no thought of revision seems entertained or desired. When the terms of Historical Geology are used, therefore, it is simply an act of convenience, as the writer pointed out in an earlier paper (Kelley, 1939). It can scarely be conceded that the terms "Carboniferous", etc., have any definite time value, yet they are convenient terms since they are in general acceptance and convey some idea at least of the stratum or strata from which the material is derived.

Sources of Material:—The most hopeful source of material for fossil mycorrhizae is in the Coal-balls which have been found and described from Europe and America. Harder fossilizations in the midst of the coal, they preserve in often intimate detail the structure of root and contained fungus from an extinct flora. Where the fungus is present in actual tissues of the host and shows structure similar to that of living material, we may feel assured that we are dealing with a mycorrhiza; but, where fungi are found in peat or otherwise, it is not so clear that they are mycorrhizal.

Fossil Phycomycetes:—Butler (1939) described "the vesicular-arbuscular or Phycomycetoid endophytes which commonly occur

in cultivated and probably other soils, forming mycorrhizal associations in the roots of many flowering plants and cryptogams, including prothalli of liverworts and some ferns." After describing these fungi, he notes the "fossil records" of fungi "of this type". Thus KIDSTON and LANGE found a fungus, *Palaeomyces Asteroxyli,* very regularly in inner cortex of *Asteroxylon Mackeii* and of basal region of stems having transitional structure between rhizome and stem, all from Rhynie Chert assigned to Early Devonian. It is not clear that evidence is afforded of any mycorrhizal structure in this fossil material, and we may note that *Palaeomyces Gordonii* is found on *decaying* stem of *Rhynia major.* BUTLER cites still further the *Protomycitis protogens* described by SMITH in 1884 from rootlets of *Lepidodendron,* assigned to Lower Coal Measures of Yorkshire; but again we do not know that there is positive evidence for considering this material mycorrhizal. SEWARD says that *Peronosporites antiquarius* is found in scalariform tracheids of *Lepidodendron* from Coal Measures. The supposed reproductive bodies may be oögonia or sporangia, or merely vesicular enlargements of hyphae. Similar swellings are seen in cells, probably of cortex of *Lepidodendron* or *Stigmaria,* from the Halifax Coal Measures. Such material would be questionably assigned to mycorrhizae.

Still more recent material comes from peat bogs, locally known as "muskegs", in Alberta through Prof. LEWIS of Edmonton; but here again there is no positive evidence of a mycorrhizal nature. BUTLER (1939) described this interesting material and decided that the fungus is the same as the "well-known vesicular-arbuscular endophyte of modern plants and with the fungus described by OSBORN and HALKET". Again, ROSENDAHL (1943) reports the same sort of fungus from three Pleistocene sites in Minnesota and refers the fungus to the genus *Rhizophagus.* The fossils came from a depth of more than 80 feet in well-borings, and after sand was washed from the matrix the material was examined. From the excellent photographs, one would think that he was looking at mould fungi; and it is stated in the paper that the fossil fungi had grown on moss leaves and coniferous needles, which are scarcely the organs in which one would naturally look for mycorrhizal fungi. Incidentally, it may be mentioned that according to ELLIS (1917) there are 15 species of fossil Phycomycetes known, and of these he mentions *Paleomyces bacilloides* as a saprophyte on fossil leaf mould.

Fossil Hepatics:—So far as we are aware, there is no record of fossil endophytes in hepatics. It would doubtless be a difficult study of a rare specimen were fossil mycothalli to be described.

Then, again, it must be realized that few students of fossils have any keen interest in mycorrhizae, and many examples of our science may languish in slide-boxes as in sarcophagi of our science. When it is realized that morphologists looked at the Hartig net in roots of woody plants for many years before realizing that they dealt with anything more than "curious thickening strips", and even today are inclined to ignore or at best to tolerate mycorrhizae, it is not to be wondered that palaeobotanists, who have even less interest, should have succeeded so well in ignoring them.

Fossil Ferns and their Endophytes:—There are three papers dealing with fossil mycorrhizal ferns. The first paper, by SEWARD (1924), deals with the fern *Tempskya* from Montana. "Some roots have lost the xylem, and the centre is occupied by a group of dark brown bodies that may be coprolites of a small insect or, in some cases, possibly escaped cell contents. Entomologists whom I have consulted have not been able to identify the oval bodies with the activities of any known boring animal: no trace of any insect has been discovered. Attention has elsewhere been called to the resemblance of these bodies to the supposed coprolites frequently found in tissues of Carboniferous plants." SEWARD figures the same sort of bodies as those figured by JANSE (1897) for *Celtis,* and the writer found similar structures in *Juglans* collected at Mont Alto, Pennsylvania.

In *Osmundites Dowkeri,* "The ground tissue cells contain traces of distinct fungal hyphae, and in many of the parenchymatous elements the cavity is completely filled with spherical vesicles; in other cases one finds hyphae in the center of the cell while vesicles line the walls. CARRUTHERS refers to these bladders as starch grains, and this may be their true nature; their appearance and abundant occurrence in the parenchyma certainly suggest vesicular cell-contents rather than fungal cells. I could detect no proof of any connection between the hyphae and bladders, and the absence of the latter in the cavities of the tracheids, favoured the view of their being either starch grains or other vacuolated contents similar to that in the cells of the Portland cycad referred to." (SEWARD, 1898).

The third paper on fossil ferns is by ANDREWS and LENZ (1943), who describe a petrified Coenopterid fern stem from the Middle Pennsylvanian of Illinois, which contains an abundant mycelium in the cortex. The fern stem, or possibly a rhizome, has been described by ANDREWS as *Scleropteris illinoiensis,* and the mycelium is found within host cells throughout the cortex although it is somewhat more abundant in the middle and inner regions. Hyphae were also found

in tracheids of the stele although they do not assume typical mycorrhizal form in these cells. Whether or not all this mycelium belongs to the same fungus cannot be stated positively. Most of the mycelium appears to be intracellular and typically endotrophic but there is some evidence that it may be intercellular as well. A considerable number of host cells contain a very dense aggregation of mycelium, while in many of the host cells infected in this way the mycelium tends to assume a nearly spherical form until finally the hyphae lose their identity as individual strands and in some cells the entire mycelial body appears as a nearly uniform amber-coloured sphere. This action may have resulted from a plasmolysis of the entire contents of the host-cell, or there is a possibility that phagocytosis has occurred. In a few of the cortical cells there may be noted a number of larger bodies varying from 15 to 33 μ in diameter, which are considered tentatively as vesicles.

Mycorrhiza of Fossil Lycopod:—One of the best known instances of fossil mycorrhizae was described by Weiss (1904). A mycorrhiza or perhaps a mycorrhizome was found in the Lower Coal Measures, the root not being associated with the plant which bore it; but the plant was possibly Lycopodiaceous and was referred to the form genus *Rhizonium* of Corda. Hyphae were found in root-hair and in epidermis but for the most part in the inner cortex, where hyphal swellings were found. The vesicles are usually empty but sometimes contain homogenous contents. "The obvious resemblance between these clumps in the fossil plant and those of recent mycorrhiza, together with the close agreement in the structure and behaviour of the Fungus in the outer layers of the cortex with those of the Fungus in recent mycorrhiza will, I think, be regarded as sufficient evidence for the conclusion that we are dealing in the case of this fossil plant with a mycorrhiza or a mycorrhizome. The Fungus differs materially in its manifestations from other cases of endotrophic mycorrhiza so far observed in fossil plants and in no case suggests that it was living either saprophytically or parasitically upon the host plant. The excellent preservation of both the Fungus and the host plant and the specialization of the cortex into two layers comparable with the 'Pilzwirtzellen' and 'Verdauungszellen' of recent mycorrhiza would suggest that, as in the case of the latter, the host plant is deriving some benefit from presence of the Fungus."

Mycorrhizae in a Seed Fern:—For the Pteridosperms or Seed Ferns there is one record of possible mycorrhiza by Ellis (1917). According to Ellis, in the fossilized vegetable remains of

the Lower Coal Measures, it is not unusual to meet with fragments of fungal threads. *Peronosporites gracilis* is very widely distributed in this horizon, the hyphae occurring in cortex of young rootlets of *Lyginodendron Oldhamium* and are not wanting in the stele, in which both hyphae and vesicles were found. The fungus was probably a parasite according to Ellis. He says further that vesicles, both terminal and intercalary, were found, and tuberous swellings. While in older plants the cortex alone is invaded, in young plants stelar cells are also infected.

Mycorrhizae in Cordaites:—One tree at least of the Palaeozoic was provided with mycorrhizae, for these structures in *Cordaites* have been described in some detail. Osborn (1909) described the roots of *Amyelon radicans,* which has been shown to belong to this group. It bears such remarkable and irregularly arranged bunches of lateral roots that Osborn examined them to discover if these bunches might correspond in any way with the root tubercles of recent plants. These lateral roots are found to have a thick cortex divisible into two regions, the inner of which contains dark cells that show evident fungal hyphae. The fungus occurs in knots of non-septate hyphae that sometimes bear terminal vesicles but there was no trace of spore formation. The conclusion was reached that this tree probably inhabited saline swamps and had bunches of coralline roots such as are known to occur in many recent plants under similar conditions. Osborn considered the relation of fungus to *Amyelon* to be in the nature of a mutualistic symbiosis.

In another study of *Amyelon,* Halket (1930) made sections of a British coal-ball and found in longitudinal sections of rootlets of *Amyelon* that they "not only showed the structure of the root-cortex but also had fungal hyphae present in its cortex, and forming a definite 'fungal zone' round the stele". The description and excellent photomicrographs indicate that the structure of those ancient Carboniferous rootlets was very similar to that of coniferous rootlets of today. Root-hairs were not as a rule developed. The diarch rootlets, which branched laterally as a result of division of cells in the pericycle, had apices which indicated that many of the rootlets had "limited growth". The (septate) hyphae were mainly intercellular but formed vesicles and arbuscles intracellularly. The author mentions, and the illustrations would seem definitely to indicate, digestion stages in cortical cells; but the vascules were never invaded. Halket considered the symbiosis to be of mutual benefit.

Summary:—There are no records of mycorrhizae in fossil Angiosperms because, so far as we are aware, there are no descriptions of fossil angiospermous root structures. Impressions of aerial organs in clay beds give us no clue to the subterranean organs; but since mycorrhizae were so well developed in the lower plants, it would perhaps not be an unwarranted assumption that they occurred in higher plants also. The general picture of ancient life that the fossil record gives us is a duplicate of the one we see today: There were forests and on the forest floor was leaf litter and mould in which saprophytic fungi lived; and the rootlets and other subterranean structures of ferns, lycopods and trees were invaded by fungal hyphae as they are today, and these hyphae produced swellings and vesicles that give the prepared sections a modern appearance. Then, too, there are "digestion stages" that indicate phagocytosis occurred in those old mycorrhizae. Mycotrophism is by no means a new process, for it appears coincidentally with the appearance of rooted plants. The explanation of mycotrophism on any developmental basis involves serious problems.

"The antiquity of fungi also raises again the question of their origin, whether they came from the Algae or from one or more separate and distinct phylogenetic lines. The sum of geological evidence appears to favor the conclusion that they have been distinct from the beginning and should not be placed in the same phylum with the algae." Wolf and Wolf, *The Fungi,* vol. 2, p. 488, 1947.

Lecture V

DISTRIBUTION OF MYCOTROPHIC PLANTS

General:—It is unknown whether plants in nature have root-hairs or mycorrhizae,—or neither; but there is enough evidence at hand to indicate that mycorrhizae predominate over root-hairs in the majority of cases. That many plants can produce root-hairs when grown under artificial conditions of greenhouse or laboratory control has been amply demonstrated, yet it is also demonstrated that these same species of plants when in their native haunts may produce mycorrhizae. Hence, almost exclusive study of root-hair plants in botanical classwork is questionably scientific, and some day Botany must revise its programme; for the attitude of traditionalism that has fastened itself upon Science is unfortunate. In the future, the Geographical Distribution of root structures will doubtless be better known; but at present something of a picture of mycorrhizal distribution may be gained from incidental references made in various papers. There are very few researches that deal directly with the subject.

Two general observations may be noted before the geographical data are presented. First, Costantin & Magrou (1926) thought that geographical distribution of symbiotic plants depends on distribution of mycorrhizal fungi: Thus, mountain plants rest ephemerally on the plains because of absence of appropriate fungi. But this idea is not very well established and awaits further evidence. Second, Wilkins & Patrick (1939) thought that "there is a possibility that the phanerogamic species may influence fungus distribution." This idea is perhaps better grounded than the former.

Since more than one half the students of our science have lived in western Europe, more is naturally known of mycorrhizae in this region than in the rest of the world.

Germany:—Botanists of Germany, earliest and chief center of mycorrhizal study, have given us records of a large part of the German flora. Chief among these studies are those of Schlicht (1888), a student of Frank. He was led to investigate herbs of his region by finding mycorrhizae on *Ranunculus acris* and he came to the conclusion that mycorrhizae are "distributed over a great range of our flora." His reports form an almost unique model for in each case he

lists the species, its habitat, and locality. Thus, he found *Lotus corniculatus* in sandy soil at Halensee, *Fragaria vesca* in forest at Negast in Pomerania, and *Myosurus minimus* in humus-rich soil at Putbus. It is refreshing to find such precision when so often a paper states in its title that it recounts "The Occurrence of Mycorrhizae in Pine", for example, when actually the paper merely tells about a few samples of one sort of pine collected in an unnamed locality. May all students of mycorrhizae pay close attention to the place and conditions of growth of their material!

A somewhat similar list of mycorrhizal and non-mycorrhizal herbs was published by HOEVELER (1892) that follows SCHLICHT'S statements rather closely. Many of the species these investigators listed as non-mycorrhizal are now known to possess phycomycetous endophytes, but 41 out of 68 investigated species were placed in this category. About an hundred other German authors tell us of various other mycorrhizal plants, and sometimes a locality is given, as, trees and herbs from East Prussia, pine from the Brandenburg Marshes, or alder from Breslau.

From all these studies, FRANK'S early (1888) conclusion seems justified that most German plants are mycorrhizal. FRANK said that in all the many hundreds of cases of cupulifers examined in forests throughout Prussia mycorrhizae were never lacking, and he said that the "Umstand, dass diese Symbiose an den natürlichen Standorten eine allgemein verbreitete, überall und an jedem Individium constant auftretende Erscheinung ist, gibt derselben den Charakter einer Anpassung der Pflanze an die Pilzthätigkeit, wobei diese von der letzteren einem bestimmten Nutzen zieht." And this conclusion was emphasized by STAHL (1900) who said that "die Mehrzahl der höheren Pflanzen, wenigstens gelegentlich, in diese Symbiose mit Pilzen eingeht."

France and the Iberian Peninsula:—France, although it stands second to Germany in number of mycorrhizal students, gives us less information about the native flora since French students have been more concerned with the problems of mycotrophy. There are few citations of locality in French accounts, and no list of French mycorrhizal plants. BOUDIER in 1876 found *Elaphomyces* about Montmorency; LECOMTE in 1887 noted beech, chestnut, oak and hazel mycorrhizal in the Vosges; DANGEARD in 1896 noted poplar, about Poitiers; MANGIN (1910) collected *Castanea* in woods at St. Cloud and Viroflay; BOYER (1915) found *Trametes* connected with tree-roots at Vallon; DUFRENOY in 1920 had collected *Adenostyles* in the beech woods of the Pyrenees at 3700′ A.T.; NICOLAS (1924) collected

mycothalli of *Lunularia* at Toulouse; while COSTANTIN has collected in the forest at Fontainebleau.

With these records, we may go on to the Iberian Peninsula where chestnut is found mycorrhizal in Portugal (CAMARA, 1907); but otherwise we know nothing of mycorrhizal conditions in these lands. MENDES D'ALMEIDA in 1908 presented a general account of mycorrhizae in Portuguese.

British Isles:—Crossing the Channel to the British Isles, we find little information on mycorrhizal distribution although a considerable number of papers on mycorrhizae have been published, especially in England. The earlier papers on the subject were published in *The Phytologist,* years 1842-1844, and localities of collection were noted, as Cotswold Hills (LEES), Lancashire coast at Southport (W. WILSON), and Southport, Kent and Sussex (SOMERVILLE); but this praiseworthy habit was not continued by later investigators. RAYNER (1911) said that her *Calluna* was common on chalk downs of the south of England where collections were presumably made; and HARLEY (1937) made his collections of beech in the Chiltern Hills. Only two English papers deal at all with distribution of endophytic structures: RIDLER (1922) cites various localities where *Pellia* mycothalli grow, while PAULSON (1923) cites birch from Epping Forest and in the following year listed certain trees as mycorrhizal in woodlands of south-eastern England, *viz. Quercus Robur, Fagus sylvatica, Carpinus Betulus, Betula alba, Castanea sativa, Pinus sylvestris* and *Taxus baccata.*

For the north-east of Scotland, I. GORDON (1936) cites 16 species of broad-leaved trees as mycorrhizal and 8 species as having no mycorrhizae; but as these eight are oaks, maples, etc., one would suppose they might be reinvestigated with profit. Strawberry plants in the Clyde Valley are mycorrhizal according to O'BRIEN (1928). The one Welsh paper (SAMPSON, 1935) deals with *Lolium,* the one Irish paper (JENNINGS, 1898) with *Corallorhiza* from the eastern Alps. The British Isles offer an almost virgin field to the student of mycorrhizal distribution.

Lowlands and Scandinavia:—Crossing back to the Lowland countries, we find little information about mycorrhizae. HESSELINK (1924) wrote on mycorrhizae of pines in afforestation of the Netherlands dunes, and there is a paper on hepatics; but future studies must tell of mycorrhizal structures in woods of Limburg or in plantings of The Bosch. In Denmark, pine is mycorrhizal in the brush-lands of Jutland (P. E. MÜLLER, 1902); and so is the im-

portant tree, *Alnus* (Bornebusch, 1914). Certain fungi are always associated with certain trees in Denmark, according to Lange (1923): thus, *Amanita muscaria* grows under conifers, also under birch, but never under beech. At Oslo in Norway, Horn (1933) found fairy-rings formed by *Hebeloma* about the bases of ten young trees of *Betula lenta,* and examination proved the tree roots to be abundantly mycorrhizal. This fungus is generally found in Norway to the limits of birch distribution. Birch, aspen, and conifers are the chief trees of Scandinavia and naturally are most studied by mycorrhizal investigators of these countries. The studies of Melin on pine, spruce, larch, aspen, and birch are justly well known; and the ecological studies of Romell involving Swedish trees and their fungi. Lihnell (1939) made an extended study of the mycorrhizae of *Juniperus communis;* Lindquist (1939) made cultural studies of spruce. Hammarlund (1923) studied the association of *Boletus* with *Larix.* An elaborate study of root development in *Betula* was made by Laitakari (1934) with ecological emphasis on soils, mycorrhizae being most plentifully developed on moorland soils and least on sandy soils. In 1920 Thesleff presented a study of Basidiomycetes of Finland; and that about completes our knowledge of mycorrhizal distribution in Scandinavian countries. Since beech forest finds its northernmost limit in Sweden, it would form an interesting study to investigate the woodlands of Skåne and compare the mycorrhizal structures with those, let us say, of French woodlands.

Baltic and Russian States:—Of the small Baltic States we know nothing of their mycorrhizae; but Voss & Ziegenspeck (1929) have made valuable studies of ericads and other native plants of East Prussia, about Königsberg. They conclude that the xeromorphy of these moorland plants is due to mycotrophy. Arcularius (1928) studied nodules of *Hippophaë* collected from the Baltic region, while Endrigkeit reported on *Allium, Molinia* and several trees from E. Prussia. One of the earliest students of mycorrhizae in Poland, Bönicke (1910), found that several members each of three families, *Ophioglossaceae, Orchidaceae* and *Pyrolaceae* are endotrophically mycorrhizal and that germination stages and cell structures may be used as distinguishing characters. The hepatic *Haplomitrium* is mycothallic according to Lilienfeld (1911). A number of exotic conifers in Poland are mycorrhizal (Dominik, 1937), and native members of *Viola* (Zabloca, 1936).

In Russia, in the Gov. Cherson among dry arid sand vegetation, fungal nodules were found on the herb *Tribulus terrestris* (Issatchenko, 1913); while in the Gov. Ekatinerinoslaw it was thought

that oak seedlings had failed because of destruction of mycorrhizae in a very wet summer (Nadson, 1908). Ganeshin (1923) found mycorrhizal connection between pine and larch, and *Boletus luteus* and *B. elegans*. That is all we can say for mycorrhizal plants in the vast extent of the Soviet Union.*

The Arctic:—Looking northward to the Arctic, one learns that perennial plants which inhabit these frigid areas are likewise mycorrhizal. In the one paper for the Arctic region, by Hesselman (1900), there are described plants collected on the Swedish Nathorst Expedition, and we learn that Arctic species of *Salix* are constantly mycorrhizal while the herbaceous *Polygonum viviparum* is thoroughly infected in both its bulbils and countless adventive roots. For the Antarctic, Johow (1889) observed that *Arachnites* from Antarctic South America is the only humus plant known from polar lands.

The Alps:—Coralloid mycorrhizae were described by Hesselman for *Dryas octopetala* both in arctic and alpine situations; and this description was confirmed by Colla (1931) for the Alps at the laboratory of La Linnaea. As early as 1888, Ebermayer had observed roots of spruce, fir and beech only in the humus layer of forests in the Bavarian Alps; while Stahl (1900) noted *Populus tremula* as mycorrhizal in alpine as well as in lowland situations, and he included a section of a couple of pages on alpine mycorrhizae. Tubeuf (1903) observed that *Pinus Cembra* lives with root fungi in alpine humus. In the Vanoise, Costantin & Magrou (1926) found structures like those reported by Hesselman for the Arctic. They say that *Salix* in Savoy has a structure identical with that in the Arctic; and from several studies they derive the generalization that mycorrhizal symbiosis is found not only in a single species in all stations of its range, but in numerous species of a genus or even genera of a family (as the *Ericaceae*) disseminated throughout the vast domain of arctic and alpine regions. They conclude that mycorrhizae play "a great role in alpine flora as well as in the arctic", and they list both ecto- and endotrophic forms. Peyronel (1937) also generalizes about distribution of alpine mycorrhizae, having studied them on the Italian side of the Alps, and at Kleinen St. Bernhard. He regarded endotrophic mycorrhizae as universally distributed in the alpine plant world and believed that members of a plant association are most closely bound to each other through symbiosis with a common mycorrhizal fungus. Malan (1938) worked with legumes in the Alps and his "results showed that in all the Leguminosae

*There is an article on a bolete of Russia, as a mycorrhizal fungus, by Vasiklov. Sovetsk. Bot. 1944(2) :21-27, 1944.

studied endotrophic mycorrhiza with hyphae of the Phycomycetoid type . . . predominated", as a Review stated; or, as the original has it: "In tutte le leguminose studiate prevalgno micorize ectotrofiche con ife de tipo ficomicetoide". Orchids have also been observed in the Alps: Beau (1920) found that in a grotto of the Maritime Alps the orchids *Cephalanthera* and *Epipactis* alone of green plants penetrated to depths of the grotto, being able to grow in subdued light by the aid of symbiotic fungi. Jennings (1898) studied *Corallorhiza* in the eastern Alps.

Central Europe:—In Bohemia, Němec wrote of mycothallic hepatics; Peklo, of various mycorrhizae; and more latterly Klečka & Vukolov (1935), of numerous congeries of trees and shrubs. Detailed investigations of one hundred eleven woody species were made in which mycorrhizae occur as constant phenomena independent of soil properties, and "it follows that mycorrhizae are a generally distributed phenomenon in woody plants." The species studied comprised most if not all the woody plants of Central Europe and a number of exotics such as *Cedrus atlantica, Thuja occidentalis,* and *Cornus florida.* It is one of the best modern studies extant. The same authors (1937) studied salt-marsh plants collected from saline soil about Neusiedler See and from Auschitz and Louny in Bohemia. The roots of *Suaeda maritima, Salicornia herbacea, Plantago maritima,* and six other species showed mycorrhizae which were identical in structure with endotrophic mycorrhizae found by the authors in woody plants. These observations coincide with those of Mason (1928) except for *Salicornia,* which was not mycorrhizal in England. Another Bohemian study, by Smotlacha (1911), indicates that certain boletes are confined to the neighbourhood of certain trees, as *B. rufus* with aspen, and he infers that mycorrhizae are oftentimes formed on a certain tree only by a certain fungus.

In an early Austrian paper, Henschel (1887) wished to upset any idea of a beneficial symbiosis and he stated very positively that presence of mycorrhizal fungi is "absolutely injurious" to spruce. Another Austrian paper deals with endotrophic mycorrhizae of *Asclepiadaceae* (Busich, 1913), an unusual group for mycorrhizal study, but as the material came from a botanical garden it tells us nothing of Austrian plants except that *A. syriaca* is not mycorrhizal. In lower Austria, *Pyrola* is endotrophic and its mycorrhizal association is obligatory (Fürth, 1920). In Hungary, Bernátsky (1900) wrote on exotics and philosophized on mycotrophy.

The Balkans:—For the Balkans, we learn that *Daphne* is mycorrhizal in the land of the Croats, at the northernmost edge of the Balkan

peninsula; and Skorić (1925) comments on the curious fact that both ecto- and endotrophic forms should be found in the same genus. From nearby Istria were collected the mycorrhizomes of the orchid *Centrosis,* used by Arcularius (1928) in his studies. The shrub Forsythia, which is native to the Balkans, is known to be mycorrhizal.

Italy:—For the Mediterranean region there are papers only from Italy, except for those by Rivett (1924) and by Dufrenoy (1917) on *Arbutus.* The Italian papers deal almost exclusively with northern Italy and leave the maqui vegetation of southern Italy for future study. Since mycorrhizae occur in chaparral of California (Cooper, 1922), a similar plant formation, it is to be presumed that they may occur also in the maqui. A considerable number of wild and cultivated plants of northern Italy have been investigated, particularly by Peyronel, who tells of the general localities of his collections, as the Val Germanasca and the Valli Valdesi in Piedmont, forests about Pisa, etc. Peyronel (1922*b*) concludes: 'L'estinza di micorize in un grandissimo numero, verosimilimente la maggiore parte, della pliante vascolari e un fatto accertato da tempo della osservazioni di numerosi riceratori."

One paper, by Ruggieri (1937), records mycorrhizae for almond in the province of Syracuse in Sicily; and Reed & Frémont (1935) say that citrus is mycorrhizal in this island. For the future, we may expect studies of root structures in scrub vegetation of Mediterranean shores, a comparison of those of the desert flora of the Mediterranean area with those in America, and studies made in the numerous islands and in the Balkans where forests still await students of our science. Several papers have appeared in recent years from the University of Pavia, notably by Ciferri and by Elisei.

Africa:—Crossing to African shores, we find endophytes in Morocco. Emberger (1924) tells of hepatics collected in this land, and Miège (1936) has a paper on potato. As for the Atlas Mountains and their *Cedrus* forests, we know nothing of possible mycorrhizae, nor do we know anything of the alpine flora of Africa. According to Stefansson, there is probably more permanent snow in equatorial Africa than in all of the Arctic lowlands, and it will be interesting to learn what effect it has on vegetation, in comparing root-structures of the Arctic and the Alps with those of the Kilimanjaro and the Ruwenzori Ranges. As for the rest of equatorial Africa, we are in entire ignorance for no botanical Livingstone has invaded dripping forest of the lowlands nor arid plateaus to learn for us what the root structures may be. Yet there are some notes pre-

served by Rayner (1938) who brought together data from several African forestry stations on growth of gymnosperms with or without soil inocula,—notes from Taganyika, Nyasaland, and Rhodesia. In South Africa, Auret (1930) wrote on the hepatic *Lunularia,* and Smith & Pope (1934) on the exotic *Eucalyptus.* For Madagascar there is a paper by Heim (1937), who says that clove trees in the east of Madagascar and on the island of St. Mary possess a *Pythium*-like mycorrhizal fungus. Three papers come from Egypt, two of them being on cotton while the third deals with several garden plants,—all mycorrhizal. Africa offers a great opportunity for original work in this field.

Asia:—Continuing with Asia, we find that the forests of this greatest of all continents, whether tropical or temperate, are unexplored by students of our science: nothing is known of possible mycorrhizae on the high plateaus or in the vast taiga, in arctic lands or in the high mountains. All the reports that come to us from the mainland of Asia are from India except that Reed (1935) says *Citrus* is mycorrhizal in Malaya. From India come two papers on *Casuarina,* which is of course not a native; from Tocklai in N. E. India Tunstall (1925) reports on tea mycorrhizae; while Chaudhuri (1925-35) tells of the hepatics. Butler (1939) found phycomycetoid infection in a number of cultivated plants in Indian plantations. And it may be noted that *Litchi chinensis* of China was found possessing short roots and intracellular infection when imported into the U.S.A. (Coville, 1921).

So much for the continent. In Ceylon, mycorrhizae are also found on tea roots (Park, 1928); while Parsons (1938) gives us notes on orchid cultivation and orchid rhizoctonial fungi from the island. For Sumatra there is a paper by Palm (1930) who said that a *Boletus,* probably *B. pallidus,* was observed to grow in association with *Pinus Merkusii* in forests of Sumatra where ground vegetation was sparse and needle litter deep and compact. From Borneo a paper by Posthumus (1937) tells us that *Leguminosae,* often in symbiosis with bacteria, are frequent in the dry savannas of the Padang Loewai in E. Borneo, taking the place of mycorrhizae of acid soils.

Java:—It is Java, however, that is the principal seat of mycorrhizal study in these great islands, for in Java are found the Buitenzorg Botanical Gardens where some of the best known students of our science have worked. Chief of all was Janse (1897), whose classic paper records presence of endophytes in selected cases through-

out the whole range of that tropical flora. In the course of a study of parasites of the coffee-tree, JANSE's attention was drawn to fungi on roots, and from that beginning he was led on to make an extensive study of roots of tropical plants. It seemed preferable to study plants from native haunts and hence almost all material was taken from the forest at Tjibodas, which belongs to the Botanical Garden and is situated on the flanks of the Gedeh volcano at an altitude of 1400-1800 m.A.T. The flora of E. Java is of an extraordinary richness and, as it was impossible to study the roots of all the plants, he decided to omit ectotrophic sorts entirely and to devote his attention to the endotrophic, mostly of large forest trees. In general, only a single species of each family represented at Tjibodas was studied; and in this logical way JANSE built up his excellent study, which nevertheless is only a preliminary one. He summarized his results in a graphic table which is here reproduced, showing the numbers of plants studied in each taxonomic group, with and without mycorrhizae:

Tabular summary of endotrophic mycorrhizae in some Javanese plants:—

	TREES		HERBS		TOTAL	
	Plus	Minus	Plus	Minus	Plus	Minus
Cryptogams	1	0	5	2	6	2
Gymnosperms	5	0	0	0	5	0
Monocotyledons	2	0	12	3	14	3
Dicotyledons	38	0	6	1	44	1
Total	46	0	23	6	69	6

Several years before JANSE published his paper, GOEBEL had written on hepatics (1891) and *Lycopodium* (1888) at Tjibodas and their fungal infection. MIEHE (1911) had called attention to vegetation on volcanic soil in Java, the pioneer plants being provided with root symbionts. He suggested that there is a significant relation between occurrence of these plants and soil conditions, naming *Casuarina, Myrica, Albizzia,* and two ericads as particularly involved. FABER (1925) confirms these suggestions, stating that all the investigated solfatara plants are mycorrhizal, the root symbiosis apparently serving for N assimilation since the soil is very poor in N. He notes among these plants two groups, one xeromorphic as the ericads, and the other more nearly hygromorphic.

Some special studies of Java plants are to be noted: TREUB (1885) reported a *Pythium* in roots of sugar cane; FIGDOR (1897) on the gentianaceous *Cotylanthera tenuis*; CAMPBELL (1907) on *Ophioglossum*; STEINMANN (1929) on mycorrhizae of *Cinchona* which, he said, is the first report for this tree; PIJL (1934) on mycorrhizae of *Burmannia* and *Epirrhizanthes.*

From the Dutch East Indies in general come these reports: *Casuarina equisetifolia* growing on coral islands of the bay of Batavia possesses root nodules like those of legumes (Kamerling, 1911). A paper on non-symbiotic germination of orchids by La Garde (1939). *Hevea* rubber trees in the D.E.I. are endotrophically mycorrhizal (d'Angremond, 1939). A method of mycorrhizal staining by Frahm-Leliveld (1941).

Japan:— This country has produced a third of the students of our science in the Orient. Earliest among them was Kusano (1911), whose study of the orchid *Gastrodia* attracted much attention; while later Hamada (1939) studied *Galeola* from the Kyoto district, these being the two orchids studied in Japan. Nodulous plants have attracted more attention, *Coriaria* having been studied by Katakoa (1930) and Shibata (1917); *Podocarpus* by Kondo (1931), Mimura (1933), and Shibata *l.c.; Alnus* by Masui (1926) and previously by Shibata (1902); *Myrica* by Shibata *l.c.* Conifers, another of the mycorrhizal favourites, attracted Masui (1926), Shimizu (1930), and Tazoye (1940), the last describing rootlets of coniferous seedlings but saying nothing of mycorrhizae. Nakai (1933) wrote on the fern, *Cheiropleuria.* Two papers only give insight into geographical distribution of mycorrhizal plants in Japan.

Takamatsu (1930) wrote on the solfatara plants in the region of Hakkoda, studying all the 28 species that existed there, a number limited by the high acidity of such soils; and he found 6 of the 28 fungus-free. These six were grasses and sedges, hydrangea and *Aletris,* and *Pteridium* which is elsewhere known to be mycorrhizal. The mycorrhizal species are *Pinus, Betula, Salix,* various ericads with other shrubs, and some herbs. Asai (1934) presented a well-organized paper in which he reported presence or absence of mycorrhizae from many plants in various habitats. He stated that mycorrhizae are absent from *Polygonaceae, Centrospermae* and close relatives: ectotrophic mycorrhizae are limited to a few families and most mycorrhizae are endotrophic. Collections were made in a tropical island, an alpine mountain of Japan, on the seacoast, fields and cultivated soil. Many grasses were found to be mycorrhizal, and a *Drosera.* Specific localities, as given by Schlicht, are not given by Asai; but he does give a good idea of root structures of a considerable cross-section of the Japanese flora.

New Zealand:—The first New Zealand paper (Cavers, 1903) on fungal symbionts was appropriately on a liverwort, *Monoclea Forsteri,* described from these pleasant islands of the South Pacific;

while succeeding papers are in a chance taxonomic order. Holloway (1920) wrote on fungal symbiosis in epiphytic prothallia of several New Zealand lycopodia, describing the infected thalli in some detail. Next may be noted some remarks attributed by Prof. Cockayne (1923) to Prof. E. H. Wilson, who thought that slow growth of pine in garden soil was due to climate rather than to any lack of microörganisms. But Walker (1931) presented a study of the mycorrhizae of *Pinus radiata,* "one of the chief exotic timber trees of New Zealand", collections of which were made in various localities in the Nelson and Canterbury districts. Miss Walker stated that no difficulty in establishing this pine had been experienced in N.Z. McKee (1941) gives us a paper on growth of spruce at Conical Hill, a mycorrhizal explanation. And from Birch (1937) came a paper on "forest fungi of significance in New Zealand" which records mycorrhizal symbiosis proved or suspected in several exotic pines, in *Betula alba* and *Nothofagus Solanderi.* Neill (1940) wrote on endophytic infection of *Lolium,* which however was not mycorrhizal since the infection was confined to the leaves. Invasion of roots was found in field grown plants but the hyphae differed from those of the leaves.

Later, Neill (1944) recorded mycorrhizae caused by *Rhizophagus* in virtually all vascular components of the New Zealand flora except in exotic pines, where it has not "been identified with certainty."

Australia:—This country has attacked the mycorrhizal problem especially from an economic standpoint. McLennan has made intensive studies of *Lolium* while Young has studied exotic conifers, the former in Victoria and the latter in Queensland. From Queensland comes also a note by Simmonds (1936) which records mycorrhizae on exotic pines and the rapid infection following acidification of the soil with sulphur. The first published mention of mycorrhiza in Queensland is said to refer to *Pinus taeda* and to date from 1928. New South Wales gives us McLuckie and a series of papers on mycorrhizae,—of *Dipodium,* an orchid which grows under *Eucalyptus; Gastrodia,* another orchid; of *Macrozamia, Podocarpus, Casuarina,* and *Eriostemon,*—in which last study Alan Burges participated. Victoria provides, in addition to the work of McLennan, a paper by Coleman (1936) on *Sarcosiphon,* a rare Thismiaceous plant associated with hazel. In the adjacent island of Tasmania, Saxton (1930) studied *Pherosphaera,* one of the *Podocarpineae.* At Penola, in South Australia, Samuel (1926) found an oat disease associated with typical endotrophic mycorrhizae and this discovery led him to a

further study: he found 27 species of legumes infected in the same way as Jones (1924) had described; roots of other crops and fodder plants, weeds and native plants were examined and a large majority were found to be infected to some extent with fungi. *Pinus insignis* and *Eucalyptus rubida* had ectotrophic mycorrhizae. All species of legumes (27) and grasses (30) were found to be mycorrhizal. Likewise in South Australia, Eardley (1932) described mycorrhizae of *P. radiata.* In Western Australia, according to Kessell (1938), "it appears to be part of the standard practice to inoculate nurseries . . . with the appropriate fungi, thus obtaining normal growth of the tree plants; the infected plants when put in the forest are said to infect the soil quite satisfactorily."

Besides these papers which give us information on mycorrhizae by states, there are several general Australian papers. Three papers tell us about mycorrhizae of pine: Cromer (1935) on those of planted *P. radiata;* Burbidge (1936) on root development of *P. pinaster* and the seasonal variation of its mycorrhizae; Ludbrook (1940) on a correlation between mycorrhizae and boron deficiency in plantation soils. Pittman (1929) described mycorrhizomes in the orchid *Rhizanthella,* and listed "mycorrhizae" for about a dozen other sorts. Fraser (1931) presented an unusual study on the genus *Lobelia,* of which two species are said to maintain an obligate relationship with mycorrhizal fungi.

Thus, in the splendid series of Australian papers much is given in regard to mycorrhizae of exotics, and something of the native flora; and there are some excellent detailed studies. Yet the student of mycorrhizal distribution finds almost a blank page for Australian native flora, for there are no records for the lower plants and a very limited representation of the higher plants. Indeed, practically all of the Australian flora is yet to be investigated for occurrence of mycorrhizae; and the same may be said for that of Africa, Asia, South America and North America apart from the U.S.A. The Hawaiian and other Pacific islands are yet to be studied for root-structures, except that for the Philippines one paper is reported (Hatch, 1937).

South America:—Several papers treat of South American mycorrhizae: They were described from several Brazilian species of *Sciaphila* by Poulsen (1886); while Macfarlane (1897) described a mycorrhiza from *Philesia,* a liliaceous plant of western Patagonia. Mycorrhizae were recorded for *Citrus* by Milanez (1940), for the first time for South America, it is said. Marchionatto (1940) has a preliminary note on the endophyte of *Lolium* in Chile; while from Chile also came the *Solanum Maglia* used by Bernard (1911). Cacao

is mycorrhizal in Venezuela (Laycock, 1945). In addition, a paper by Berggren (1887) treated of austral conifers, including *Araucaria,* but no localities were given. An article has been published recently by Castellanos on nodules of alder in mountains of Argentina (Lilloa 10:413-416, 1944). To sum up the matter, there is not yet a single paper devoted to mycorrhizal distribution in South America.

West Indies and Central America:—For the West Indian islands four studies may be cited: Johow (1885) published a paper on West Indian saprophytes belonging chiefly to the genera *Burmannia* and *Apteria.* Mycorrhizae of sugar-cane in San Domingo were studied by Ciferri (1928), who has also published on mycorrhizae of the Burmanniaceae (1946). A fuller description of cacao mycorrhizae is given by Laycock & Dale (1945). A brief note by Palm (1930) on pine in Guatemala is all that can be said for mycorrhizal distribution in Central America. With botanical facilities available in the Panama Canal Zone and in Puerto Rico and with the example of the Buitenzorg Gardens before them, it would seem that the Americans might match the splendid contributions from the Dutch East Indies with some good studies of mycorrhizae in the American tropics.

North America:—In North America there are no mycorrhizal studies whatever to report from Mexico or from Alaska, while from Canada come three papers,—a neat study of *Taxus* from Quebec (Prat, 1934); a short note by Lewis (1924) on *Picea* of Alberta; and a citation of raspberry (*Rubus*) by Berkeley (1936). In other words, North America, apart from the U.S.A. is yet to be explored for root structures of plants.

North-eastern U.S.A.:—There is not very much known of mycorrhizal distribution in the U.S.A. New England, oldest center of learning in the country, has told us nothing of the subject, except that Ames (1921 *et seq.*) described "mycorrhizae" for some orchids while Stokey (1924) reported fungal infection of *Lycopodium* prothallia in western Massachusetts. *Epigaea* from Connecticut provided Barrows (1936) with material for her studies. For the Middle Atlantic States there are two papers by Henry that tell us of the Wading River region of Long Island (1934) and of Butler County in western Pennsylvania (1933). For Long Island he lists all the pines and junipers, birch, chestnut, oaks, maples and ericads, showing that a cross-section of a pine-barren area exhibits all the woody plants as mycorrhizal. In western Pennsylvania, in a deciduous forest area, he reported 60 species of woody plants as mycorrhizal,—a large

proportion of the native flora. *Myrica carolinensis* from the coastal area of New Jersey was described as mycorrhizal by HARSHBERGER (1903). In an unpublished paper prepared in 1930, KELLEY listed as mycorrhizal 160 out of 172 spp. of woody plants investigated in the Middle Atlantic States.

Southern U.S.A.:—There is only one paper on mycorrhizal distribution in the Southern States of the American Union, a paper by McDOUGALL (1928) on the 16 spp. which he observed in the Great Smoky Mountains of North Carolina and eastern Tennessee. Three of these species he considered non-mycorrhizal, namely *Leiophyllum* and *Rhododendron* which are ericaceous, and *Sassafras,* which KELLEY finds to be mycorrhizal. His other species are conifers, cupulifers, magnolias and hickory. Besides this paper there are isolated observations: Pine is mycorrhizal in North Carolina (ASHE, 1915; COBBE, 1916); while southern pine (presumably *P. palustris*) is termed mycorrhizal by PESSIN (1939) and HUBERMAN (1940), both authors saying that the mycorrhizae are abundant. PESSIN in 1928 reported 4 spp. of pine mycorrhizal at Bogalusa, Mississippi, and mycorrhizae abundant on seedlings grown at McNeill, in the same state. The orchid *Tipularia* collected in South Carolina contains an endophyte (CLIFFORD, 1899); the valuable pecan (*Hicoria*) bears mycorrhizae in Georgia (WOODROOF, 1933); the exotic *Casuarina* is mycorrhizal in Florida (MOWRY, 1933); while the orchid *Zeuxine strateumatica* of S.E. Asia is now established in peninsular Florida (PORTER, 1942). ATKINSON (1892) noticed galls on *Ceanothus* collected in Alabama.

Central U.S.A.:—Turning next to the mid-portion of the U.S.A., active centers of mycorrhizal study are found. In Indiana, DOAK (1927) presented a list of 21 mycorrhiza-bearing species of plants collected about Lafayette, mostly trees but with a few herbs, including the fern, *Adiantum.* In Illinois, LESSMAN (1928) listed "a new form of ectotrophic mycorrhiza" on *Quercus bicolor.* At Urbana, Illinois, McDOUGALL & LIEBTAG (1928) examined 145 of the 183 spp. occurring in the university woods and found that mycorrhizal fungi occurred on roots of 93 spp. PFEIFFER (1914) found *Thismia* with endophyte on prairie near Chicago. For Michigan, DUTHIE (1908) presented a list of mycorrhizal tree species but the list cannot be followed since no scientific names are given. A detailed study of ten species of native plants growing in bogs of the Huron River Valley led TRANSEAU (1906) to consider their "mycorrhiza" to be detrimental, but as they were bog plants perhaps the structures were

actually pseudomycorrhizae. Prothallia of the fern *Botrychium virginianum* collected in Grosse Isle contained an endophyte according to JEFFREY (1898). Two papers on connection of sporophores to tree roots come from Michigan (KAUFFMAN, 1906; PENNINGTON, 1908), KAUFFMAN noting mycorrhizae on oak, sugar maple and *Celastrus* at Ann Arbor. For cultivated plants, A. H. SMITH (1930) described endotrophic mycorrhizae for various fruit trees about Ann Arbor. FREEMAN (1904) made studies of *Lolium* at the University of Minnesota. In an unpublished paper, KELLEY described root-endings for woody plants of the Kawishiwi Ranger District of the Superior National Forest (northern Minnesota), all the species proving mycorrhizal. In Iowa, LOHMAN (1927) made a valuable study of the "occurrence and nature of mycorrhizae in Iowa forest plants", collections having been made in central and northwestern Iowa. Seventy individual plants were studied (16 being ferns) in 40 species of which 20 had a constant root endophyte, 5 an occasional, while 15 were fungus-free. For Missouri, there is a paper from a forest nursery where MILLER (1938) studied influence of mycorrhizae on growth of short-leaf pine seedlings (presumably *P. echinata*). From St. Louis come several papers on exotic orchids.

Rocky Mountains:—In the Rocky Mountains, mycorrhizae are found on 8 spp. of trees and 3 spp. of *Cercocarpus* (MCDOUGALL & JACOBS, 1927); and ectotrophic mycorrhizae on certain trees of the Uinta Basin of Utah are described by HENRY (1936), who also recorded absence of mycorrhizae from 7 spp. A survey of northern Colorado flora bearing mycorrhizae was made by THOMAS (1943), listing 21 families.

Pacific Coast:—On the Pacific Coast, studies come only from California except that there is one paper from Oregon that deals with nursery trees. The three principal species of the scrub vegetation or chaparral of Jasper Ridge, *viz. Adenostoma, Quercus* and *Arctostaphylos* are mycorrhizal in sand and clay as well as in humus (COOPER, 1922). *Sarcodes* was collected at San Bernardino by OLIVER (1890), and presumably MACDOUGAL's (1900) collections were Californian since the plant occurs nowhere else. More lately (1944), MACDOUGAL & DUFRENOY report on *Aplectrum, Corallorhiza* and *Pinus Torreyana.* Smaller roots of celery were heavily infected with fungus in delta peat soil (RAWLINS, 1925); while REED & FRÉMONT (1934) found mycorrhizae generally present on *Citrus* in California.

LECTURE VI

MYCOTROPHIC PLANTS AND THEIR ENVIRONMENT

Soil as a Mycotrophic Habitat:—There is perhaps no satisfactory definition of soil. If soil is defined as the "unconsolidated upper few feet of the earth's crust", then some mycorrhizae do not occur in soil at all, for one may find them in pockets of humus formed by decaying stubs on trunks of living trees. Such occurrences are not uncommon with *Acer rubrum* growing in swamps of the eastern U.S.A. where the living tree will develop "necklace-beaded" mycorrhizae in pockets of humus on its own trunk from a root-branch developed by the trunk. Or, seedlings of other species, which have been termed "pseudoepiphytes", may develop in such situations. CORDEMOY (1904) found that aerial roots of *Vanilla* form mycorrhizae in rotting supports that are supposed to hold the plant up; and the same thing may be seen even better with pepper vines. Again, in a forest one may find dozens of spruce or *Tsuga* seedlings growing on a partly decayed log or stump; and occasionally one finds a sapling that started in such a situation and later extended its roots down into the soil, the stump or log meanwhile rotting, leaving the sapling standing as it were on stilt roots. All of these examples show that plants can grow for some years in a flourishing condition without any contact with mineral soil. If it is necessary for trees to have mycorrhizae in order to gain inorganic salts from the soil, they must win the salts vicariously for they have no direct contact with the soil, yet the seedlings flourish.

In contrast, there are plants growing and producing mycorrhizae in pure mineral soil which, as in MÖLLER's (1902) sand, showed no trace of humus. Or, mycorrhizal plants are found in agricultural soil where humus and mineral portions are mixed together, although in such situations the vesicular-arbuscular mycorrhiza is more likely to be found. It may be said, then, that mycorrhizae are formed wherever rootage organs grow in contact with appropriate fungi.

Mycorrhizae and Soils:—Consequently, mycorrhizae are found in a variety of soils. VON TUBEUF (1903) said: "Die Mycorhiza findet sich auf Moorboden, im Waldhumus, auf nährstoffreichen Lehm—und gedüngten Ackerböden und selbst im Bleisande des

Eberswalder Kiefernbödens"; and SARAUW (1904), after reviewing the evidence, said that in general: "la formation des mycorhizes n'est influencée que d'une manière quantitative et non qualitative par les différentes sortes de sols." But PRAT (1926), in a study of *Taxus,* concluded that the nature of the soil seems to exercise an influence on branching of the roots. HARLEY (1937) agrees in that he says the form of mycorrhizae in beech and extent of infection is correlated with soil type. KELLEY (1941) studied mycorrhizae of *Pinus virginiana* in four soil series,—Chester (granitic), Conowingo (serpentine), Dekalb (sandstone), and Sassafras (Cretaceous gravel); and he found characteristic differences in each of the soil series. The mycorrhizae were coral-branched in sandy soils and racemose or elongate in clay soils, while pseudomycorrhizae predominated in wet clay soils. In droughty sands were found necklace-beaded mycorrhizae, caused by intermittent growth. A. MÖLLER is said to have found different sorts of mycorrhizae in different soils about Berlin (HENRY, 1903).

FRANK had supposed that mycorrhizae are found only on humus soils but are absent from *sands* and sandy soils. MÖLLER (1902) thought, on the contrary, that pines produced mycorrhizae in sand rather than in humus. In France, VUILLEMIN (1890) "a constaté aussi que le terre sabloneuse de bruyères est favorable aux mycorhizes." PESSIN (1928) records pine mycorrhizae from Norfolk sandy loams and Orangeburg fine sandy loam in the southern U.S.A.

Calcareous soils are usually considered unavailable to mycotrophic plants since most mycorrhizal fungi prefer an acid substratum. But *Calluna* is rather an anomaly since it grows on chalk downs which are rich in mineral constituents but poor in lime. RAYNER (1921) found that the mycorrhizal fungus grew well even in a strongly alkaline extract of *p*H 8.0; but *Calluna* developed normally in presence of fungus (but with exclusion of bacteria) only on Ca-poor soils. RUGGIERI (1937) found almond mycorrhizal on calcareous soils of Sicily. Pine does not thrive on alkaline soils, and it is possible that some of the difficulty with establishment of pine on prairie soils may have been due to the alkalinity of the limestone soils of the region. YOUNG (1938) corrected alkalinity in nursery beds by S applications.

Solfatara soils of volcanic regions present special conditions. MIEHE (1918) had drawn attention to the fact that mycotrophic and bacteriophagic plants occur in numbers on solfatara soils, and supposed that they were excellent pioneers inasmuch as they are able to fix atmospheric N. FABER (1925) came to the same conclusion: he said that these soils are characterized by Al-content, N-poverty, acid content, and high temperature; and that solfatara plants are adapted to these factors. He found both xeromorphic and hygromor-

phic plants included but decided that there was no "physiological dryness" of the habitat, nor was there any lessening of transpiration in these plants. They assimilate Al so greatly that they could be termed "Al-plants". All sorts of mycotrophic conditions were described by Takamatsu (1930) for the solfatara soils at Hakkoda, and the mycorrhizal structure was similar to that of mycorrhizae from forest soils.

Humus:—Whatever name is placed on the complex of substances usually denominated "humus", it is manifest that these substances are determinate in the distribution of mycorrhizae. Two principal forms of humus,—raw-humus and mull—are associated with "ectotrophic" and "endotrophic" mycorrhizae respectively; and these in turn are related to certain edaphic conditions, especially water. Hence there is an interplay amongst organic detritus, microörganisms and moisture that determines existence of mycorrhizae. This organic detritus is undecomposed; and when it is broken down with formation of mineral salts, it ceases to be humus. It is futile, therefore, to speak of absorption from humus of water and mineral salts. It is equally futile to say that mycorrhizae make use of humus: what mycorrhizae use is the protoplasm of invading fungi. These invading fungi utilize humus through the soil portion of their mycelium, either directly or through the mediation of microörganisms; and the humus has ceased to be humus when these organisms have made use of it . . . Hence mycorrhizae never take in or "absorb" mineral salts; and how they acquire water is, to the best of our knowledge, still a matter of conjecture.

Humus is naturally formed by the partial breaking down of organic matter, chiefly vegetable, by the action of microörganisms. Earlier workers, such as Nikitinsky (1902), had shown that humic substances could not be used directly by higher plants but that they were broken down by bacteria and fungi into simpler products that could be used. In his well-known studies of humus-formation, Falck (1923) used the term "Mykokrinie" to describe the chemical changes involved in the decomposition of forest duff; and by this process fallen branches, dead leaves, etc., are transformed into humus and finally into "mineral salts" that the higher plant can utilize. In Sweden, where Melin (1925) has studied so intensively, the forest soils may be divided into a *mull* type wherein there is a more or less rapid destruction of plant residues resulting among other things in nitrate formation; and this type has a rich herbaceous ground-cover; or a *raw-humus* type resulting from a less rapid destruction with scarcely any nitrification while the organic matter remains in an

ammoniacal state: this type has a moss and lichen ground cover. In raw-humus MELIN visualized a struggle between fungi and trees for N, and the tree provided with mycorrhizae could compete with soil organisms for that N. In mull soils, he found mycorrhizae (*i.e.*, obviously ectotrophic) poorly developed. Yet ENGLER (1903) had supposed that mull but not raw-humus is available to mycorrhizal fungi. The nature of the soil plays an important part in distribution of these fungi (PEYRONEL, 1921); and also on fungal form since in soils poor in organic materials rhizomorphs develop while in humus a disperse mycelium is produced. Then, not only fungal but mycorrhizal form is influenced by humus (BJÖRKMAN, 1940), for Type C of MELIN, formed by *Boletus* spp., was found on occasional pine plants in "mor" and sand (humus mixed with sand in a volume ratio of 1:2). An interesting line of work is brought out by MAGROU: "In the cultivated field, manure is capable of elevating the osmotic pressure of the soil solution and, in consequence, that of the sugar of the plant, beyond the level at which tuberisation might commence." (Ann. d. Sci. nat., Bot., XI, 4:97-102, 1943).

Two ideas about humus have governed students of mycorrhizae, *viz., (1)* that humus by its decomposition provides salts to the plants growing in it, and *(2)* that humus buffers the soil by absorbing deleterious substances. In other words, the influence of humus is considered to be either *chemical* or *physical*.

The first idea is the older. FRANK, in his experimental work recorded in 1888 and 1889, indicated that fungi living in humus obtain their nutrient from it and change over the humus into directly assimilable N compounds or ammonia. For beech, MÜLLER (1886) concluded that the tree lives on the remains of its own vegetative activity, a peat being built up under the tree in which mycorrhizae live. Not only beech but spruce and fir live thus in the Bavarian Alps (EBERMAYER, 1888), the tree using ammonia salts directly and also mineral salts derived from the humus layer. Of these salts, coniferous litter was found to contain more N, deciduous litter more K and P. (FERRY, 1887). By removal of leaf-litter, the fungi are deprived of their normal food-supply and are transformed from mycorrhizal into parasitic fungi (DELACROIX, 1897).

The physical influence of humus on mycorrhizal development has been adverted to by several earlier workers, as *e.g.* HOEVELER (1892) who noted a rich branching of the mycorrhizal root system in humus, while SHIMIZU (1930) thought that humus determines mycorrhizal form in pine. But more recently the idea has been current that in humus there are inhibitory or deleterious substances which interfere with growth of plants, and that possession of mycorrhizae enables

certain plants to grow in these soils. According to Freisleben (1935), "The beneficial action of fungi on the growth of Ericaceae . . . does not rest on a direct influence, as through the excretion of growth-promoting substances, but on an inactivation, destruction or absorption of the inhibiting materials. It is to be supposed that in natural soils also the root fungi and the soil fungi which enter as components of a peritrophic mycorrhiza, have a similar significance for the Ericaceae." Again, Rayner's (1944) Wareham experiments indicate presence of a toxic substance in this infertile soil and in a note, Neilson-Jones (1940) adduced some experimental evidence to prove the hypothesis that emerged from Dr. Rayner's experiments in Wareham forest: "The hypothesis was put forward that the local infertility on the area is due to toxic residues formed during decomposition of organic detritus by micro-organisms; and that the effect of the compost is to provide a substrate which, by altering the serial activities of the different soil micro-organisms, modifies the chain of reactions constituting soil decomposition; with the result that the final residues, instead of being toxic, are favourable to the growth of the trees, to the mycelial growth of the fungi associated with them as mycorrhiza-formers, and to the establishment and free-functioning of a normal and balanced mycorrhizal relationship."

Mycorrhizae as Soil Indicators:—Bernátsky (1900) considered mycorrhizae as indicators of poor although well-aerated soils except in the case of *Alnus*. Certain generalizations can perhaps be made: A coralloid mycorrhiza is associated with a raw-humus forest soil; racemose mycorrhizae of light colour mostly come from mineral soil; bushy-branched rhizothamnia are characteristic of sands; while pearl-necklace mycorrhizae are found in droughty soils. In mulls the preceding sorts are not likely to be found, but endotrophic structures prevail.

Mycorrhizae in the Soil Profile:—Mature natural soils present a profile that is considered to exist in three "horizons", *viz.* the "A" horizon, or zone of leaching and extraction of salts and colloids by percolating waters; the "B" horizon, or zone of concentration of these materials; and the "C" horizon, or zone of subsoil and rock fragments where there is no visible concentration of leached materials.

There is not enough data extant to formulate any comprehensive picture of the occurrence of mycorrhizae in the soil profile but certain cases are known. In northern coniferous forests where raw-humus develops, mycorrhizae are developed chiefly near the surface in the uppermost A horizon. Masui (1927) figured a soil profile for "woody

plants" in which mycorrhizae are located in a layer above the conducting roots. For forest trees of central Europe, Klečka & Vukolov (1937) state that mycorrhizae are developed most richly and best in "the middle root depth". Scully (1942) found greatest concentration of small roots in the A_1 horizon and greatest numbers of dead roots in a lower horizon. But trees may form mycorrhizae at some depths in the soil: Pecan mycorrhizae may be formed at 30 inches depth (Woodroof, 1933), while mycorrhizal roots of *Pinus densiflora* were found by Mimura (1933) at 10 m. depth. On shallow infertile soils certain nut-trees have the roots confined to the upper levels and the rootlets are almost entirely turned into mycorrhizae, whereas on deep fertile soils the mycorrhizae are widely and deeply distributed (Schuster, Stephenson & Evenden, 1944). Frank (1887) had stated that in German forests the mycorrhizae occur in the uppermost 1.5 cm. of humus while at lower depths there are fewer although they may be found at ½ m. depth. Yeates (1924) also found, in the New Zealand *Podocarpus,* that the roots are mostly at the surface in an organic layer, a condition which, he said, is possible only in a rain forest. In Finland, Laitakari (1934), in an elaborate study of root development in *Betula,* found that horizontal roots occurred at depths of 2.8 cm. to 31.1 cm.; on moranic soils the depths were greatest (av. 20 cm.) while on water-logged soils they were least (ca 8 cm.). Vertical roots penetrated to depths of over 2 m., being deepest in clay. *Fagus* in England, according to Harley (1937), shows variations in root systems with depth of soil: In shallow soils on chalk the whole substrate is colonized while in deep plateau soils the roots are fairly evenly distributed in upper layers of mineral soil. In podsols and semi-podsols, fine roots are restricted to litter and humus layers.

Herbs and dwarf shrubs apparently have superficially placed mycorrhizae. *Calluna* root system (Rayner, 1911) is confined to the first 12 inches of soil; while Burgeff (1932) stated that hemisaprophytic organs of orchids are found in the uppermost layers of soil.

Soil Texture:—Effect of soil texture on root development was neatly shown by Ter-Sarkisow (*cf.* Kirchner, 1908) for *Pinus sylvestris,* 4 month old seedlings in pots showing the following root development:

	Number	Length
Sand	363	713
Loam	181	420
Humus	54	179

Pine is successful when planted in grey sand of Australia (Cromer, 1935); but Laitakari, already cited, found mycorrhizae most plentifully developed in moorland soils and least in sands. Pecan forms spreading clusters of mycorrhizal short-roots in light or sandy soils but fan-like clusters in firm-textured soils such as the heavy red clay subsoils of northern Georgia (Woodroof, 1933). Influence of texture in humus on mycorrhizal form was neatly described by Mangin (1910): In soil formed of leaves which are superimposed and compressed the mycorrhizae are often distichous with their branches in the same plane; while in contact with débris of cupules and fruits their form is more or less complicated and branches of the mycorrhiza are oriented in all directions and more or less pelotonized, dependent upon size of the space in which it develops. In duff the mycorrhizal short-roots of *Populus* were found to be clustered into nodules while in sand they were betuloid in type, being ordinarily dark in colour except where growth is renewed (Kelley, 1937). Where a layer of humus overlaid clay, Kelley (1941) found seedling pine with mycorrhizal short-roots in the humus but rootlets that had penetrated into the clay beneath were transformed into pseudomycorrhizae. A somewhat similar case was reported by Frank (1888) in that a beech seedling had mycorrhizae in the upper layers of soil (to 20 cm. depth) while deeper occurring roots were fungus-free. Frank also noticed that roots were much more richly branched in humus while in poorer soil layers the roots assumed an elongate form. Perhaps the latter were pseudomycorrhizae, but that term and concept had not been formulated in Frank's day.

Soil Moisture:—Mycorrhizae are found only under optimum conditions of moisture; that is, optimum conditions during the period of growth. Thus, mycorrhizae are absent from aquatic plants so far as known, yet they may be found on marsh plants (Mason, Osborn), or on semi-aquatics like *Oryza* (Peyronel, 1922). They seem absent or less abundant under bog conditions where pseudomycorrhizae are more frequently found but are common enough in meadows and in moist ground in general. On the other hand, they may be found in soil that is very dry and even on desert cacti (Asai, 1934, Johansen, 1931) but they are present under arid conditions as reserve organs and were formed while moisture was ample for growth. But too much drying may result in death of the mycorrhizae, and Paulson (1923) described an interesting case from Epping Forest, England, where an unusually dry summer killed the mycorrhizae, thus cutting off supplies of nutrient to the trees and paving the way for entrance of secondary parasites and saprophytes by which many of the birch trees

that composed the forest were destroyed. Yet in the case of pine, CROMER (1935) found that, while drought caused collapse of the cortex of absorbing roots, it did not affect that of the mycorrhizae. Besides physical dryness of the soil, physiological dryness must also be considered; yet FABER (1925), in a study of volcanic soils of Java, decided that occurrence of the same association in the crater of the volcano as well as lower down on the sides in wet volcanic soil may not be explained through the hypothesis of "physiological dryness" of solfatara soil but it is the result of individual nutrient conditions on young solfatara soils.

Certain mycorrhizal plants such as *Obolaria* and *Orchis spectabilis* are found only in moist shady places and thus indicate a relationship to soil moisture. This relationship was noted as early as 1889 by JOHOW. Dependence is probably on the mycorrhizal fungi which can extract nutrient materials from moist humus and duff but not from dry materials. On the basis of soil moisture one might separate the *mesic* mycorrhizal plants from the *xeric* ones such as ericads, conifers and certain cupulifers, the former being chiefly endotrophs and the latter ectotrophic. However, it must be noted that VOSS & ZIEGENSPECK (1929) have shown that xeromorphy in ericads may be due to physiological conditions resulting from mycotrophy rather than to dryness of soil.

It must be observed that mycorrhizae are chiefly developed in the uppermost A horizon in what is naturally the driest portion of the soil, at least on well-drained sites. For this reason there is a selective action on mycorrhizal plants, the mesic species being confined to sites where minimum soil moisture for mycorrhizal development is higher than for xeric species. Certain anomalies in plant distribution can thus be explained: Thus, *Pinus virginiana* grows on hills of Pennsylvania and Maryland but is absent from adjacent sand flats of the coastal plain of Delaware where oak woodlands flourish. It was found by experiment that seedlings of the pine transplanted to open sands of the Delaware area could not withstand desiccation of the summer dry season; but where pine is watered or grows in favourable lowland (there is a colony of *P. Taeda* near Newark), the tree is able to grow in spite of dry seasons. In the same way, *Orchis spectabilis* grows on shaded north slopes of Pennsylvania woodlands but never on sunny south slopes where xeric ectotrophs thrive. BOUDIER (1876) had noticed that *Elaphomyces* is found on south and east slopes more than on north and west slopes at Montmorency in France.

Another fact that must be taken into account is that rainfall on an area is by no means uniform in distribution nor regular in occurrence; and these irregularities have a consequent influence on mycorrhizal

development. After a rain there is a rapid root growth but as available soil moisture lessens in amount growth slows and may cease, to be renewed with the next rainfall. Mycorrhizae therefore are not necessarily structures of a steady growth that finally comes to a definite end, but growth can be renewed. After a rain there is rapid mycorrhizal growth but as available moisture lessens, growth is retarded, to recommence at the next rainfall. Every student of mycorrhizae has seen old brown or even black mycorrhizae that have split their mantle and protruded a new white mycorrhizal apex. With some plants the periods of growth and quiescence are marked by constrictions or rings, and the mycorrhiza assumes in consequence a beaded appearance. Even in winter a warm rain starts new mycorrhizal growth and one finds abundant white mycorrhizal root-tips.

Soil Solution:—Much is known of the composition and physics of the soil solution but its relationship to actual plants growing in native habitats is problematical. For, if plants are nourished through a mycorrhizal apparatus located in the uppermost A horizon, of what particular interest to them is a soil solution in the mineral B horizon? Scientists, with that habit so ingrained in the human race, have gone into the utmost minutia of research regarding the soil solution, but they have never gone to the trouble to find out whether roots of naturally growing plants actually come into contact with this solution. Even for the mycorrhizae that do occur in the B horizon it is not known what significance the soil solution has for them because the mycorrhiza is buffered, so to speak, by fungal structures that more or less isolate the mycorrhiza from the soil. Hence the whole question of soil solution and mycorrhiza is largely conjectural, and because of its character the various theories of mycotrophy are conditioned.

Some of the more recent studies have thrown incidental light on the soil solution-mycorrhizal relationship. For some years Melin has emphasized the importance of N salts to mycorrhizal plants as indicated by laboratory tests. He has found that the simpler N compounds, such as asparagin, can be utilized but that more complex compounds, as peptone and nucleic acid, are used with difficulty. Harley (1937) found with reference to beech that if the fungus supplies N to the tree, it does not overcome low N content of the soil, and vigour of the beech tree is more attributable to soil variations than to variations in infection of roots; yet infected roots had a greater N content than uninfected. In the case of "fairy rings", Guinier (1937) found that ammonia content of the soil was markedly higher within the ring and grass found here was dark green. Also, in coniferous forests the ammonia content of the soil was greater immediately beneath the

sporophores of certain hymenomycetes which form ectotrophic mycorrhizae with roots of trees. GUINIER supposed that benefit of the fungi to the higher symbiont consisted in accumulation of ammonia in the soil. MITCHELL (1937), in experimental studies with coniferous seedling beds treated with various N, P, and K combinations found that the benefits attributable to mycorrhizae, like their distribution in nature, vary inversely as the concentration of readily available mineral nutrients in the soil. As to what these mineral nutrients may be, a paper by CHANDLER (1941) indicates that decay of leaf litter in a central New York hardwood forest returns Ca to the soil in greatest amount, N in the second greatest, followed by P, K and Mg. MCCOMB (1944) indicates P rather than N as incentive to good growth in conifers.

Soil Reaction:—Anyone who has worked with soil reaction tests knows the difficulties of securing an accurate reading for that highly buffered colloidal thing called soil; and if he has studied the relation of plants to soil *p*H, knows further the wide tolerance most plants show for h.i.c. He is not surprised that BIRAGHI (1936) reports cereals growing in soils of *p*H 5 to 8, and he has sympathetic understanding for the report that *Pinus radiata* was planted in grey sand of *p*H 6.18.

Long ago MELIN (1925) stated that optimum conditions for fungi of pine and fir are provided by *p*H values between 4.0 and 5.0 and he noted with interest that this observation accorded well with observed *p*H values for middle and northern Europe recorded by HESSELMAN. HENRY (1933) found trees and shrubs mycorrhizal on soils of *p*H 5.0 in Butler County, Pennsylvania. For *Pinus Strobus,* MCARDLE (1932) reports a *p*H of 6.0, a little lower for spruce. Germinating seed and young seedlings of *P. echinata* cannot survive in culture media having a soluble Ca content of approximately 500 p.p.m. or more and a *p*H value of approximately 6.5 or more, or having either of these characteristics. This condition was evidenced by behaviour of seed in greenhouse cultures and of seedlings in nursery beds. With *P. caribaea* in Australia, YOUNG (1938) concluded from experiment that "The efficiency of the mycorrhiza is increased with increasing acidity up to *p*H 4.7 and thereafter is adversely affected." For orchids, BURGEFF (1932) found the optimum values lying between *p*H 5.0 and 6.0; while LAGARDE (1929) said that h.i.c. is of the greatest importance in germination, growth being best at *p*H between 4.8 and 5.2; and above 6.0 no germination took place.

It is evident that soil reaction affects the fungal symbiont rather than the higher plant because the latter is virtually isolated from the

soil. The soil is merely an anchorage medium for the higher plant and the fungus is its body servant that makes contact with the soil. It is the fungus that benefits from acid reaction and is limited in its *p*H range. Modess (1941), in his investigations of mycorrhizal fungi, found that all investigated fungi produced acid solutions. Optimum growth occurred with the *Amanita* spp. at the *p*H range of 3.5-4.5; with *Paxillus Prunulus* and the *Boletus* spp. (with the exception of *B. variegatus*) at *p*H 5.0 or somewhat above this value, relative to *Lactarius delicosus* and *Rhizopogon roseolus* at *p*H 5.5-6.0. A species of *Mortierella* isolated from *Empetrum* made best growth at *p*H 2.77-4.0 (Hasselbaum, 1931).

It may be observed, however, that Henry (1936) records five ectotrophs growing in Utah above the aspen zone where soil is neutral or slightly acid; and Auret (1930) found the mycothalli of *Lunularia* growing in slightly alkaline soil of South Africa. Ridler (1923) also reports *Pellia* in England on soils of *p*H 6.8-7.0.

The Use of Free Nitrogen:—The several studies relative to fixation of atmospheric N by mycorrhizae may be summarized by stating that if such fixation occurs it is in too small amounts to be of consequence to the mycorrhizal plant. Melin (1922) found that fungi associated with mycorrhizae of *Pinus sylvestris* and *Picea Abies* can in no case fix atmospheric N; and in 1925 he stated that there is no fixation of free N in mycorrhizae of trees examined. Möller (1906) had found that dichotomons mycorrhizae of *Pinus montana* are of no use to the tree in fixing free N; the fungus of *Empetrum* is likewise unable to use atmospheric N (Hasselbaum, 1931); the same is true of the fungus of *Monotropa* (Francke, 1934); and also of *Mycelium radicis Fagi A* (Aali, 1923). But Rayner (1922*a*) claimed that certain strains of *Phoma* isolated from ericaceous plants can use atmospheric N and she said that *Aspergillus* and *Penicillium* are similarly capable but in varying degrees. She backed Ternetz (1907) who had published similar statements. Furthermore, Neilson-Jones (1928), experimenting with a fungus isolated from *Calluna,* decided that the "plant can obtain nitrogenous supplies from the air, probably in the form of molecular N, in sufficient amount to prevent the advent of any symptoms of N starvation." The volumes of culture solution tested were 50-100 cc., and the amounts of N fixed, from 0.00004-0.00384 gm. But Addoms (1931) decided that if atmospheric N were fixed by *Phoma radicis* isolated from cranberry (*Oxycoccus*) plants, it was in amounts too small to be of service to the higher plant.

As to composition of the soil air in general and its effect on mycorrhizae, the author knows of no studies except that Laing (1923)

noted deficient aeration and oxidation of peat soils affects distribution of mycorrhizae. This is all the more remarkable because work of LUNDEGARDH and others would seem to indicate that soil atmosphere might have a profound effect on the mycorrhiza and associated organisms, especially through an heightened CO_2 content. The great importance of optimum CO_2 supply in tuber formation has already been indicated by MOLLIARD (1920), tubers failing to form in its absence. It may be that in the future the soil air will be shown of more importance to the mycorrhiza than some of the soil influences which are now stressed.

Soil Temperature:—As with soil air, there are no direct studies on influence of soil temperature on mycorrhizae. But it is well known that soil temperature does not fluctuate to the same extent as air temperature; and in woodlands where the soil is blanketed with a layer of duff it is partially insulated from fluctuations of air temperature. A woodland with heavy leaf litter is so well protected from frost that the ground may not freeze all winter and in consequence the mycorrhizae are not destroyed as they often are on freezing. In contrast, a woodland that lacks a protective leaf litter freezes and thaws repeatedly and only certain plants, especially deep-rooted ones, survive. Again, through freezing and thawing many seedlings are heaved out of the ground whereby certain species are prevented from establishment in a habitat which would otherwise be suitable for them. Yet freezing does not necessarily destroy mycorrhizal plants for it has already been seen that such occur in arctic and alpine situations. CHAUDHURI (1935) stated that the endophytes of hepatics studied can withstand very low temperatures and even an exposure to 0° for four weeks did not kill any of them. A great number of mycorrhizal fungi seem benefitted by low temperature but in nature mycorrhizal fungi exist at many varied temperatures (MELIN, 1925). In the Japanese orchid, *Galeola,* the fungus is dominant when its optimum soil temperature of 25°C prevails while the host is more active under the more congenial conditions of the colder months (HAMADA, 1939). Yet high temperatures do not prevent mycorrhizal development, although presence of mycorrhizae in the tropics does not necessarily indicate toleration of high soil temperatures since these may be comparatively low and steady in the rain-cooled, shaded forest. But mycorrhizae of cactus are certainly exposed to extremes of soil temperature and show the hardiness of the mycorrhizal association.

The temperature of the soil may undoubtedly be changed by action of microörganisms, and a soil with a rich microflora should be a warmer soil and more favourable to winter survival of seedlings than

a sterile one. Greaves & Jones (1944) have suggested that addition of manure to soil may add new microörganisms and modify the soil temperature.

Altitude:—The production of mycorrhizae at various altitudes has been studied especially by Costantin and his associates, the effect of altitude being considered due to air temperature and thus paralleling effect of latitude. In 1926 Costantin & Magrou observed similarity of mycorrhizae of *Dryas octopetala* in the Alps and in the arctic as described by Hesselman. Hitherto only ectotrophic mycorrhizae had been observed in such situations but now endotrophic mycorrhizae were found widely distributed in the Alps. Annual plants are absent from the Arctic and rare in the Alps, but the annual *Gentiana campestris* was found to have an endophyte which, however, underwent a "brutal phagocytosis". The authors came to an important tentative conclusion that if essentially mountain genera are found sporadically on the plain, their stations rest ephemerally because the mycelium which is transported accidentally at the same time as the seed does not reproduce itself. Later (1934) these same investigators, with associates, grew potato seed at 1,400 m.A.T. and obtained some plants with infection, some without; but at 550 m.A.T. all the plants died without producing a tuber. Since potatoes are not ordinarily grown in these alpine places, the authors concluded that potato can form mycorrhizae with fungi already present in such situations. These results are aligned with the theory that the mycorrhizal habit in alpine plants commenced with a chance association of fungus and root, forced beneath ground by inclement weather. Whatever value this theory may have, it seems better established that there is an optimum altitude for mycotrophy. Bouget had studied potato since 1901, and in 1922, with Bonnier, discovered the *law of optimum altitude,* which was not published until rediscovered and published by Lebard in 1931 (Costantin, 1936). Lebard & Magrou (1935) state that, through three seasons' experiments it was shown that there is an altitude where yield of potato is maximum, decreasing above or below.

Light:—Light can affect mycorrhizae only indirectly since they are not ordinarily exposed out of the soil. But illumination does affect the vigour of the host plant, and the production of ionizable substance in the host tissues. Björkman (*cf.* Romell, 1944) has studied the effect of light on seedlings and it is stated that mycorrhizae are formed in weak light (1/16 or sometimes 1/8 full sunlight), but under greater illumination there were better seedlings with more mycor-

rhizae. Naturally under optimum light exposure photosynthesis is carried on most favourably and the host is accordingly better provided with assimilate. BJÖRKMAN finds a connection between assimilation and mycorrhizal formation, for which the reason is given in the last chapter of this book.

Phenology:—With reference to seasonal aspects of mycorrhizae there are a number of observations made incidentally in the course of other studies. Early in the history of our science, R. HARTIG (1886), who was no friend to the concept of mycotrophy, asserted that tree roots are free from fungi in summer and that mycorrhizae are present only in autumn and winter; while McDOUGALL (1914), upon whom HARTIG's mantle has to some degree fallen, claimed that mycorrhizae are annual, being formed in summer and persisting through the winter. A. B. FRANK (1888) countered HARTIG's statement by saying that "die Mykorhiza zu keiner Jahreszeit ihren Pilzmantel verliert". The mycorrhiza, he said, is formed in the earliest youth of the plant and, like all absorbing roots, dies when it has exhausted its soil locus. Mycorrhizae can exist at least two years, probably much longer. These statements were an amplification of his 1885 assertion that mycorrhizae have a limited life-span, some being lost while others are being replaced; and it seems evident that mycorrhizae are able to live several years. MÖLLER confirmed FRANK by stating (1890): "Als Beweis dafür führe ich an, das ich bei Material, welches im Januar gesammelt war, gleichwie bei solchem im Juni sammtliche Entwicklungszustände und in gleicher Verteilung gefunden habe". The fungus grew out simultaneously with the tuberous mycorrhiza of the pine studied, in summer rapidly but at other seasons as the cold permitted.

For the rebuttal, McDOUGALL & JACOBS (1927) stated that at 7,100′ A.T. on Mt. Logan, Utah, only dead mycorrhizae of the preceding year were found on *Pseudotsuga mucronata.* Above 7,000′ on Mt. Logan and at 10,000′ on Mt. Washburn in the Yellowstone Park, only dead mycorrhizae were found. New mycorrhizae can be formed only when new rootlets are being developed and mycorrhizal fungi are active, and these conditions seem to obtain in the latter part of the growing season.

It will be recalled that BÜSGEN (1901) did some cultural work on ash, beech, maple, oak, and spruce, to learn more of the disputed question of periodicity of root growth. He found that in Germany best growth occurs in June and October with little growth occurring in July and August. In March there are numerous roots growing, also in November and December. In conifers a winter rest is indicated by browning of the root-tips. GOEBEL, in the *Organography,* calls at-

tention to the fact that some trees, as *Tilia europaea,* have greatest development of roots in fall while in oaks the greatest development is in spring. Other observations indicate similar generic differences: Thus, Prat (1926) found that in *Taxus* the long-roots grow throughout the whole season with varying rapidity although cold lessens activity and causes modification of the apex. Most of the absorbing rootlets cease growth completely in winter, at which time the cortex dies from the apex and exposes a red surface, while growth recommences in

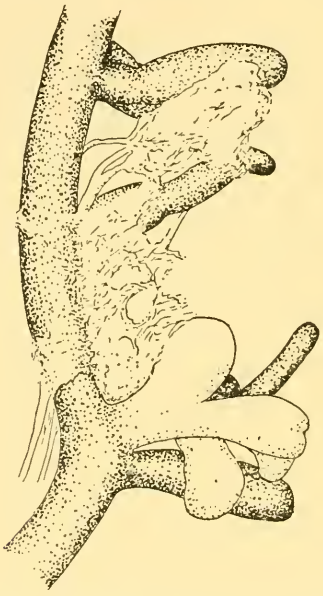


Fig. 4.—Renewed growth of mycorrhiza-bearing mother-root of *Pinus Strobus,* new white mycorrhizae being formed amongst old dark ones, with some rhizomorphic investing mycelium also indicated. Collected at Baltimore, 26 February 1930.

spring. In pine, Rayner (1934) says positively that the mycorrhizae are annual and only in exceptional cases is growth renewed. *P. Banksiana* is completely dormant in winter in Minnesota, the roots growing from April to October (Kauffman, 1945). Again, McArdle (1932) stated that mycorrhizae of spruce and pine are formed mostly in September to November inclusive and that they are usually dead by spring. Yet Preston (1943) found that pine mycorrhizae did not appear to be "strictly annual", and several instances were noted where

they had achieved renewed growth at the beginning of the growing season by bursting through the fungal sheath. With Pennsylvania trees and shrubs, mycorrhizae are present every month of the year but particularly in late summer and autumn (Henry, 1933). On deciduous trees of Scotland mycorrhizae are present every month from November to March (Gordon, 1936). Still other observations are to be recorded: Pecan mycorrhizae are to be found at all seasons in Georgia (Woodroof, 1933); in *Vitis* it is a mistake to speak of the dying of all roots in autumn, for only those formed in spring die while those formed in autumn persist through the winter into spring (Rives, 1923); in *Citrus* seasonal variation was found, mycorrhizae being best developed in the spring growing season (Reed & Frémont, 1935). In beech in England, the time of most rapid root growth (chiefly spring and autumn) is marked by appearance of numerous uninfected roots (Harley, 1937). This period is followed by one of infection of the new roots. The shallowest chalk escarpment soils are characterized by a short spring period of growth and infection; in deepest escarpment soils the spring growth persists longer, root growth and infection going on together and being interrupted only by drought. Infection is never complete and many uninfected roots are present in spring and summer. In very acid plateau soils, roots are formed near the surface and growth occurs in an upward direction in spring, incompletely decayed litter of the previous autumn being colonized by uninfected roots. In April and May infection takes place rapidly, while in early summer it is nearly complete.

For herbaceous plants there are various reports. In liverworts the fungus was found occurring in autum (Schacht, 1854); in *Pyrola* Stahl (1900) found mycorrhizae also in autumn but not in spring; while Endrigkeit (1937) said that plants of *Convallaria* and *Maianthemum* are almost completely fungus-free in spring. Orchid roots collected in September were uninfected (Costantin, 1926) while Beau (1913) stated that roots formed in *Spiranthes* at end of the flowering season are infected from the soil but not from old roots. In *Galeola* the symbiont invades the cortex during summer and autumn and ingestion proceeds through the winter until the following summer (Hamada, 1939).

Cromer (1935) had noticed that mycorrhizae of *Pinus radiata* renew their growth after rain. According to Paulson (1924) during drought of even short duration mycorrhizae are desiccated and thereby killed. "Mycorrhiza does not revive after being destroyed by lack of moisture and does not reappear on the return of copious rain until new rootlets have been developed and they in their turn have become associated with a fungus. . . . Observation of roots after heavy

rain, which followed dry weather, has been sufficient to . . . conclude that new rootlets followed by a complete change to mycorrhiza have developed within ten days."

As to phenology of internal anatomy, several observations may be cited: In *Hippophaë,* Arcularius (1928) said the fungus grows best in summer and there are new vesicles present in winter. In *Vitis* (Rives, 1923), vesicles appear at the end of the season, in August and September. In *Orchis,* fungal digestion occurs chiefly from autumn into winter (A. Fuchs, 1924). In *Fraxinus,* Kelley (1943) found phagocytosis occurring in April and May, in Pennsylvania.

Mycorrhizae in Relation to Habitat:—Apart from influence of soils on mycorrhizal form, more recent studies have been directed to influence of habitat as a whole on mycorrhizae. It is obvious that environmental influences of the habitat react first on the fungus, as indicated by Curtis (1937): "There is an apparent correlation between ecological habitat and fungus type, rather than between orchid species and fungus." In conifers the possession of mycorrhizae seems dependent on edaphic conditions (Dominik, 1937), and the more mycorrhizae are developed the better the growth. Naturally, conditions that favour the fungus result in a greater development of mycorrhizae.

In his review of soil fungi and root infection, Burges (1939) considered the soil flora with its microhabitats; and the relative abundance of fungi, which is one-thirtieth that of bacteria but greater in numbers than that of any other group. The biological groups of fungi present in the soil the author considers as (*a*) root parasites, (*b*) casual parasites and mycorrhizal fungi, (*c*) facultative parasites and primary saprophytes, and (*d*) true soil fungi. The last group comprises those of a "humus type", the second group being most difficult to study and some seem to be obligate parasites.

The term "microhabitat" graphically expresses the situation of a mycorrhiza, for it is in a little cosmos of its own. Here it is subject to the inorganic and biological influences of the immediate neighbourhood, the "rhizosphere" as it has been called. Hiltner is said to have originated this term for the space about a root which is subject to root excretion, in which he thought there is an aggregated microflora. But Kürbis (1937) pointed out that fungi live in and on tree roots and separate the root from the purely rhizospheric fungi. Consequently Jahn (1934) invented the term "peritrophic mycorrhiza" and defined the peritrophic fungus as one that lives in an outer zone, mantling the root, between soil-portion and root-epidermis. Ordinarily considered saprophytes, they bear a definite relation to the root. He said that in many cases endo-, ecto- and peritrophic fungi are present

in the mycorrhiza at the same time; or, the cortical hyphae may be neither parasitic nor endotrophic but peritrophic; and the peritrophic fungi may become dependent on the root plant. JAHN supposed that the function of rhizospheric fungi is to change the h.i.c. of the rhizosphere that it will correspond to the most favourable concentration for optimum permeability of the roots. In an experiment to determine whether it is the H- or the Ca-ion that is active, he found that several fungi cultured from the rhizosphere caused heightening of the h.i.c. of the culture solution without addition of calcium carbonate, but with such addition the *p*H changes were less but nevertheless were in an acid direction. With calcarous fungi there was better development on addition of $CaCO_3$ than without it. It had been early suggested (KUNZE, 1906) that there is not a simple relation between root-secretion and mycotrophy but that the higher plant makes use of the decided soil "ausschliessenden" action of fungi. So KÜRBIS decided that the fungal flora of *Fraxinus* are not necessarily mycorrhizal, but surround the root with acidity. He found that microörganisms were greater in numbers in the rhizosphere than in root-free soil; also, that seedlings of *Fraxinus* dwindled and died in sterile sand but waxed in unsterilized or inoculated soil.

Salt Marsh:—Two special habitats are to be considered, the salt marsh and the prairie. A salt marsh, with its high osmotic coefficient because of relatively large salt content, one would not suppose to be favourable to mycorrhizae, yet two papers record characteristic presence of these structures in it. According to MASON (1928), mycorrhizae were found in such common coastal plants as *Plantago maritima, Aster tripolium, Glaux maritima, Armeria maritima* and *Glyceria maritima,* but no mycorrhizae were found in *Salicornia europaea, Triglochin maritimum* and several others, including *Juncus.* But KLEČKA & VUKOLOV (1937) found mycorrhizal symbiosis in the small roots of *Juncus Gerardi, Salicornia herbacea, Suaeda maritima* and *Triglochin maritimum* which duplicated that of endotrophic mycorrhizae of woody plants. The material was collected from saline soil about Neusiedler See and from Auschitz and Louny in Bohemia; and the authors thought it very interesting that the fungi endured an high osmotic pressure in root cells of these species. We would like to be assured that these salt-marsh soils were truly saline, for our experience with the New Jersey marshes indicates that such soil is not necessarily salty.

Of 14 halophytes collected on the west coast of Sweden by FRIES, six bore thamniscophagous mycorrhiza which contained arbuscles, vesicles and hypertrophic nuclei. (Bot. Not. 1944:255-264).

Prairie:—The other habitat to be considered is prairie. Strictly speaking, prairie is a special sort of meadow once found in the central United States but the term is now loosely applied to non-forested lands throughout the more northern portion of the Mississippi Valley. Virgin prairie is now virtually extinct throughout the area and the soils have been changed by agricultural practice. Since trees were absent in the prairie area, except along water-courses and on some rougher lands at time of discovery by white men, it has been supposed the mycorrhizal fungi were absent from the prairies. Yet it appears obvious that prairie grasslands existed simply because tree growth was excluded by fire and difficulties of ecesis, and recent studies in Iowa indicate a rapid spread of oak-scrub over former prairie lands to the annoyance of the farmer. "Harrison County (Iowa) vegetation was used by Shimek . . . to support his thesis of climax prairie in Iowa, yet 30 years later *Quercus macrocarpa* is spreading so rapidly on the less intensively farmed lands of the country as to constitute a serious economic problem". (McComb & Loomis, 1944). Apparently these trees have no difficulty in ecesis. The author, while living on the Iowa prairies, has personally seen how readily bur-oak becomes established wherever prairie sod is uprooted.

But Hatch (1936) stressed a reputed absence of mycorrhizal fungi from prairie soils, meaning by "prairie" apparently what is otherwise known as "dry prairie" or "steppe". He noted from the literature that "16 nursery and plantation failures have occurred in widely separated regions of the world" due to "lack of a biologic factor in the soils". He secured, through friends, some "prairie soil" from Wyoming for his experiments.

As a matter of fact, Wyoming is several hundred miles west of the prairies; it is five thousand feet higher in altitude; and it has a different climate. The name, Prairie, may not be applied indiscriminately to all grasslands. The plains of North America, the pampas of South America, the steppes of Asia, and the veld of South Africa are all grasslands; but they are none of them prairies.

In this Wyoming soil Hatch grew seedlings of *Pinus Strobus* and found growth poor and unthrifty when mycorrhizae were absent but on inoculation with pure culture fungi of several species growth became good. N, P, and K determinations of the seedlings were made after 10 months growth, showing marked increase in the absorption of N, K, and especially P by the mycorrhizal plants. Hatch believed the evidence was conclusive in showing that the pine seedlings grown in this soil did not obtain sufficient nutrients to support normal growth when mycorrhizal fungi were absent; and he repeated his conclusion in a paper published the following year. Rayner (1937), ignoring

the question of "prairie", commented on HATCH's work and remarked that there is some doubt as to whether the greater acquisition of N by the mycorrhizal seedlings in HATCH's "prairie" soil experiment is related solely with the more efficient absorption of nitrates and his claim that peptone and nucleic acid can be absorbed directly by the roots of pine seedlings is not discussed from this point of view. In a paper by McCOMB (1938, also 1943) the claim is made, based on experimental data, that differences in pine seedling development in a forest tree nursery on old agricultural land in the prairie province (Iowa) are due to disparities in the amounts of available P, and that mycorrhizae are the means of enabling the seedlings to absorb this element at a sufficiently rapid rate for normal growth. Thus HATCH and McCOMB stress P but a writer in the Annual Report of the Wisconsin Agricultural Experiment Station (1942) said that inoculation of evergreen seedlings with suitable mycorrhizal fungi, particularly *Boletus felleus*, greatly improved growth and survival on prairie soil. The evidence obtained indicated that the mycorrhizal fungi rendered the K present in the soil more readily available to the seedlings.

That mycorrhizal fungi are absent from at least certain prairie soils is asserted by ROSENDAHL & WILDE (1942), who found such fungi in cut-over forest lands of central Wisconsin but "invariably absent" from adjacent prairie soils. McCOMB & LOOMIS (1944) also report a sharp difference in microflora between forest and prairie soils. HARVEY (1908) asserts an absence of fungi from prairies; yet it must be noted that PFEIFFER (1914) found *Thismia* mycorrhizal on the prairies at Chicago; and WILKINS & PATRICK (1938) presented a paper on the fungi found in grasslands about Oxford. WHITE (1941) regarded mycorrhizal fungi as beneficial, and suggested that mycorrhizae exert a specific growth-promoting effect upon forest seedlings, the absence of this stimulus being a major factor in the poor growth of trees on mycorrhiza-free prairie soils. But the majority of cases of poor growth of pine in the U.S.A. are apparently not associated with mycorrhizal deficiency (LATHAM, DOAK & WRIGHT, 1939); and in Indiana a failure that was so associated was more easily corrected by fertiliser than by inoculation. "Even in new conifer nurseries in the Prairie States growth is usually satisfactory . . ." Again, it must be noted that JONES (1924) said of endotrophic fungi of legumes in western America that no field, no matter how recently reclaimed, is free from infestation and that but few mature leguminous plants are uninvaded by mycorrhizal fungi.

A great difficulty with the question of occurrence of mycorrhizal fungi in prairie soils is, that the subject has never been investigated.

There is not a single paper devoted to an analysis of the subject and not a dozen references in the literature. The reputed absence of mycorrhizal fungi from prairie soils is simply a dictum mundi that has been adopted trustfully as an axiom; whereas there are several facts against it. Thus, trees and presumably root fungi have occurred from time immemorial along the numerous watercourses which traverse the prairies; trees and shrubs flourish along streets of innumerable prairie towns and about tens of thousands of prairie farmsteads. These woody growths have a hard battle against desiccating winds and temperature extremes in the trying climate of the region, but hardier species thrive. It is true that conifers often fail in prairie soils, but it is possible that the failure may be due to alkalinity of the soil, an alkalinity that possibly may be connected with the fact that the prairies are very generally underlaid by limestone, although prairie soils are not residual. And then it must be noted that trees spread rapidly into the prairies when the sod is broken. As to the Plains which lie west of the prairies, aridity is a potent influence on plant development, and establishment of trees in these droughty soils must always be conditioned by the water supply as well as by other "soil factors".

Soil Inoculation:—On the assumption that necessary mycorrhizal fungi are absent from certain soils on which trees are to be grown, the practice of soil inoculation with these fungi has arisen. Thus Rayner (1934) found that seedlings which grew poorly on a sterile heath were greatly benefitted by application of humus which "must contain active mycorrhizas of the species free from any abnormalities of structure and from contaminations of such pseudomycorrhizal fungi as can be identified". Probably there were "active mycorrhizas" in the transplants made to various treeless regions in those benighted days before the science of mycorrhiza flamed so brightly. Thus, Leonard Flemming, a pioneer in afforestation in South Africa, says nothing of inoculating the soil when he planted thousands of seedling pines on the high veld where trees had never grown before. It was water that the pine-trees craved and when he supplied their need the trees flourished. But in Australia, exotic conifers needed for softwood plantings in the Brisbane Valley often failed to grow at the Yarraman nursery, and on examination it was found that the roots lacked mycorrhizae (Young, 1938). (In passing it may be remarked that Frank in 1894 had raised the question whether the needful fungi were present in all soils used for plantations.) On inoculation of the seed beds with the proper mycorrhizal fungus, mycorrhizae were formed and the seedlings became thrifty. Moreover, it appears to be part of the standard practice to inoculate

nurseries in Western Australia with the appropriate fungi, thus obtaining normal growth of the tree seedlings (KESSELL, 1938). The inoculated plants when put out in the forest are said to infect the soil quite satisfactorily. RAYNER (1938) gathered together various reports on soil inoculation from nurseries and plantations, particularly from the British Empire. In northern Rhodesia it was found that *Pinus halepensis* only amongst several exotic pines made any growth beyond the seedling stage without soil inocula, whereas inoculation with soil from a southern Rhodesian *P. radiata* plantation caused remarkable stimulation in growth in several spp. of pine. In Nyasaland, all species of pine observed except *P. longifolia* and *Araucaria* failed to grow without inoculation. At Buitenzorg in Java, *P. Merkusii* is completely dependent on the presence of mycorrhizal infection for normal development, inoculation resulting in vigorous growth and rapid spread of infection from plant to plant. In New Zealand, inoculations of *P. radiata* with *Boletus*-infected soil gave positive results (whatever that means), the control plots remaining free from infection. In India, *Casuarina* flourished after inoculation whereas controls died within three years. *Caragana* became established in Canada after use of soil inocula. At a new forest tree nursery in Iowa, pine seedlings failed to grow unless they developed mycorrhizae (MCCOMB, 1943). On the other hand, S. A. WILDE remarked in a recent review that "99 percent of all practicing foresters will not have to lose any sleep over the problem of mycorrhizal inoculation."

Compost Studies:—But soil inoculation with mycorrhizal fungi does not necessarily lead to mycorrhizal formation because the soil itself may be unfavourable to such formation even though the appropriate fungi are present. Thus, in RAYNER's heath soils mentioned in an earlier paragraph, it was the inhibitory effect of the soil that prevented mycorrhizal formation. RAYNER therefore initiated studies of "organic composts" in relation to growth of young trees. Her general conclusion after several years' experiments is that an increased supply of nutrients plays a relatively insignificant part in improved fertility of the soil studied, induced by addition of composts. In the soil are substances deleterious to growth, but their action is obviated by addition of compost although addition of the equivalent amount of salts had no effect. RAYNER considers that the striking effects on tree growth brought about by composts on natural soils do not depend to any extent upon the addition of nutrients, but are directly associated with qualitative changes in the humus constituents and with the biological activities related with these changes. They may also, possibly, be associated with the presence of growth-promoting substances in

individual composts or produced in the soil as the result of fungal action. The chief biological change in relation to fertility of the soil is production of toxins, according to BRIAN (1945), especially of "fungistatic organic substances" produced by Penicillia. The chief toxin appears to be gliotoxin, which has been found highly toxic to mycorrhizal fungi. BRIAN suggests that the toxicity of Wareham soil may be due to accumulation of gliotoxin and other antibiotic substances.

Valuable as Dr. RAYNER's (1944) studies on the Wareham area undoubtedly are, the results will of course be applied with caution to other areas. Results obtained with a very unusual soil existing at low altitude but high latitude under an oceanic climate will not necessarily be applicable to all other areas. As evidence in point, the paper by LINDQUIST (1945) may be cited, in which it is stated that "larger and better-colored seedlings" of *Pinus resinosa* were grown on a duff-peat mixture than on a compost-peat area. Again, composting often produced abnormally crooked roots (MUNTZ, 1945).

A study of the organic matter of forest soils led ROMELL (1938) to a new theory of mycotrophy. In experimental work in a spruce forest in Sweden he sank sheet-iron shielding, one foot high, in a poor stand of spruce, surrounding two quarter-hectare plots. One plot, being covered with blueberry bushes, was mowed with the scythe while the other plot was untreated. The author states that a marked effect resulted, for the vegetation on the plots became thriftier and greener, and retained its foliage longer in autumn. ROMELL considered the effect due to killing of the tree roots or of mycorrhizae and their associated fungi by trenching, the organic matter thus killed becoming "green manure" for the remaining vegetation. Also, root competition of the trees, and fungal competition, was stopped. Numerous sporophores of the supposed mycorrhizal fungi were formed outside the plots while practically none were formed within. ROMELL thought that these experiments show a fundamental physiological difference between litter-decomposing and mycorrhizal fungi, the latter being practically unable to break down dead organic residues under conditions prevailing in nature. He points out the value of trenching experiments in mycotrophic studies, since laboratory experiments show merely what is physiologically possible but not what is ecologically important.

LECTURE VII

MYCOTHALLI AND MYCORRHIZOMES

General Character:—Mycothalli and mycorrhizomes are ordinary liverwort gametophytes, fern stems and orchid rhizomes that possess endophytes. Most of these structures in nature appear to be invaded with fungi, for apparently most thalli and prothalli that are not actually in water are mycotrophic, and most rhizomes likewise. Here again the fortuitous character of the symbiosis is seen, since apparently the fungi simply grow into these structures as into a part of the environment; and there is nothing evidently obligate about the relationship.

Mycothalli in Liverworts:—Their structure is detailed for *Pellia* by RIDLER (1922): In *Pellia* no plants were found entirely without infection and usually the endophyte occurs in a definite zone along the thickened median portion towards the ventral surface of the thallus and in the rhizoids. Infection from the soil is presumably through the rhizoids. Within the thallus, penetration of the cell-walls seems effected mechanically; the hyphae are swollen where their growth is arrested by cell-walls, and they are constricted by passage through them. The liverwort seems to exert some control over the fungus and limits its invasion as stated, to a definite region in the thallus. Here the hyphae form arbuscles or bushy-branched structures which later degenerate into sporangioles or little rounded bodies that are insoluble in usual reagents. Formation of arbuscles stops further growth of the fungus and this phenomenon caused BERNARD (1909) to term it an "immunity humorale". The effect of the fungus in *Pellia* is very marked for protoplasmic content of invaded cells of the thallus is killed, chloroplasts disappear and cells ultimately become brown in colour. Starch disappears from cells of the thallus on entrance of the fungus and is replaced by oil. When the sporophyte is invaded (the thallus is of course the gametophyte) the contents of the cells are wholly or partially absorbed. The fungus invades the region of the sexual organs but does not grow into them.*

*According to PEYRONEL, the *Jungermanniaceae* are infected only by mycomycetes. On poor soil, infestation dwindles with decrease of light (Nuovo Gior.Bot.Ital. 49:362-382, 1942).

Infection of Mycothalli:—Infection seems to take place always through rhizoids and is thus reported by all workers. Kny (1879) said that "In numerous root hairs (*sic*) of *Lunularia* (from the university greenhouse) it was observed that a great part harboured thread structures. In a series of cases these were sterile fungal hyphae which branched hither and thither". "Seven cultures of *Calypogeia* from very different habitats about Hilversum showed almost all rhizoids attached to substratum infested while aerial hyphae were fungus-free." (Garjeanne, 1903) Fungal hyphae penetrate rhizoids of *Marchantia* and *Lunularia,* especially where plants grow in humus (Cavers, 1903) : hyphae were found in rhizoids of *Lunularia* in South Africa (Auret, 1930) : in Italy, Bergamaschi (1932) found in *Fegatella* and in *Lunularia* that non-septate hyaline hyphae invaded the rhizoids and passed into underlying cells; while Chaudhuri (1935) found hyphae in rhizoids of all Indian liverworts investigated. An endophyte penetrates rhizoids in *Sewardiella* of southern India (Chalaud, 1932). In *Zoöpsis* of Java, the rhizoids frequently harbour hyphae which form pelotons and refractory granular material, perhaps albuminoid. Divers other hepatics from the same forest present the same endophyte (Janse, 1897).

Limitation of Endophyte:— Limitation of the endophyte to a definite portion of the thallus seems general. In New Zealand liverwort, *Monoclea Forsteri,* every thallus possessed a sharply defined mycorrhizal zone consisting of 2-4 layers of cells densely filled with branching fungal hyphae (Cavers, 1903). This zone is confined to the thicker median portion of the thallus and extends to within a short distance of the growing point. Hyphae pierce the cell-wall and branch out in the cell cavity, the nucleus of the infected cell grows in size and often becomes enveloped by a tuft of short hyphal branches and sometimes the chloroplast becomes similarly enveloped, suggesting in appearance a lichen. On some of the hyphae are formed large spherical vesicles. In *Lunularia cruciata* the fungus is confined to a definite zone below the assimilating tissue (Auret, 1930) ; it occurs also in the rhizoids and amphigastria but does not penetrate the gemmae-cups and archegonia. The fungus consists of branched septate hyphae with granular contents giving rise to vesicles, arbuscles and sporangioles which conform with the general type of endophytic fungus found in a great variety of higher plants. All plants, infected or uninfected, are green and apparently healthy. Nicolas (1942) found in *Lunularia* two sorts of infection: (*1*) confined to a band which runs the length of the mid-nerve parallel to lower surface and removed from it by several layers of immune cells rich in starch: (*2*) In other, male,

thalli the fungus is localized in cells throughout the thallus. Sterile thalli were destitute of mycelium and NICOLAS thought that presence of fungus is necessary to fructification.

EMBERGER (1924) also found hyphae in *Lunularia cruciata* occupying a large band separated from the lower surface by several layers of cells; the chloroplast tissue is never invaded. Inconstancy of infection, he thought, negated the hypothesis that infection is necessary to formation of sexual organs; and the association seemed simply accidental. It was supposed by NĚMEC (1899) that mycothalli are general in *Jungermanniaceae* but rare or absent from *Marchantiaceae.* We have already seen that infection is common in *Lunularia;* it also occurs in *Marchantia* and a number of other European hepatics according to GOLENKIN (1902), who found that in all cases the fungal hyphae are confined to the compact ventral tissue; and infected cells, though they retain nuclei and protoplasm, never contain starch or chlorophyll. Thalli of *Marchantia nepalensis* on sand and clay at Lahore, India, contained a fungus limited to a zone beneath the air cavities, and branched and interwoven in the cells (CHAUDHURI, 1925). Chlamydospores were sometimes found. In this and other Indian liverworts, infection is localized in regions definite for each species (CHAUDHURI, 1935). *Conocephalus* is similar to the preceding, as described by BOLLETER (1905) who found the thalli often turned red upon infection; but in alpine situations the thalli turned red without infection,—another fact in line with COSTANTIN & MAGROU's idea that refrigeration parallels the action of mycotrophy.

Digestion of Endophyte:—In his description of mycothallism in *Pellia,* MAGROU (1925) said that the fungus degenerates about the archegonia or the sporogonia, which organs seem to exert an inhibitory influence on its growth. The endophyte exhibits all the structures characteristic of mycorrhizal fungi,—large non-septate hyphae, arbuscles, sporangioles and multinucleate vesicles, the contents separating into uninucleate cells. Digestive structures were also described by GARJEANNE (1903) from thalli of Netherlands liverworts,—haustoria and hyphal coils (Knauel); and under influence of the latter the cells disorganized. Immersed clots were found in a number of liverworts by MILDE (1851), while NĚMEC (1904) found clots in *Calypogeia* coincident with degeneration of mid-hyphae: they disappear before death of the thallus. Many vesicles were formed in tissues of *Conocephalus* but few in *Lunularia* (BERGAMASCHI, 1932). CHALAUD (1932) figures vesicles and arbuscles in *Sewardiella.*

Tuberous-thickening of the stem of *Fossombronia* with which a fungus was always associated was noted by HUMPHREY (1906); while

Chalaud (1932) found tuber-formation in *Sewardiella* "in all respects like the stem of other Metzgerias, especially *Petalophyllum* and *Fossombronia.*"

Denis (1919) found an endophyte in the chlorophylless thalli of *Aneura,* and compared them to similar thalli known amongst lycopods.

Mycothalli in Fern Gametophytes:—A description of the mycothallus in *Opioglossum pendulum* is given by Lang (1902). Tissues of the young prothallus are parenchymatous throughout, cells of the lower portion contain an endophytic fungus while those of the upper portion are free from it. Infection is usually through a rhizoid. Superficial cells of the prothallus contain only infecting hyphae, the

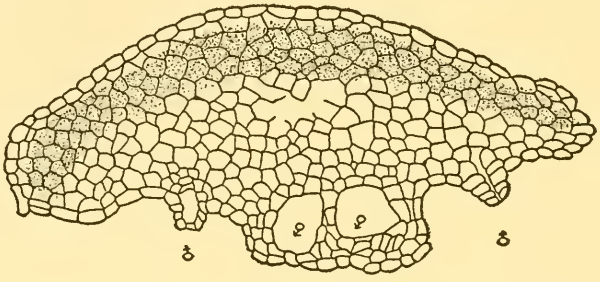


Fig. 5.—Section through an older mycothallus of *Botrychium obliquum.* Shaded portion indicates the extent of region occupied by endophyte, vicinage of reproductive organs being uninvaded (*Redrawn from* Campbell, Ann. Bot. 35, pl. viii, fig. 12, 1921).

fungus otherwise being confined to internal tissue. In older regions of a branch the fungus occupies all the cells except for a superficial zone of 1-2 layers. A number of vesicles are formed in a cell, often close to the nucleus, while other cells contain thick coiled hyphae. Plastids and chloroplasts occur in cells occupied by the fungus. The European *Ophioglossum vulgatum* mycothallus was described by Bruchmann (1904). Infection is directly through the epidermal cell-wall and hyphae spread through the mid-portion of the prothallus but the outer cells are always fungus-free. Innermost cells are also fungus-free and contain starch. Nuclei of infected cells increase in volume while hyphae coil in the cells and form an irregularly shaped structure: vesicles occur in older portions of the prothallus. The infected portion forms in effect a cylinder which is particularly well developed about the sexual organs.

Campbell (1907) in general confirms Lang's description of prothalli in *Ophioglossum,* and in the appendix of his *Mosses and*

Ferns gives a longer description; he found infection also in prothallus of *Osmunda cinnamomea.* In 1908 he stated that he had found an endophytic fungus normally present in green prothalli of several *Marattiaceae, Osmundaceae* and *Gleicheniaceae.* The endophyte consisted of large, branched non-septate hyphae which are strictly intracellular. Vesicles and apparent digestion stages are described, and in figure ten there is shown a vesicle near an intact nucleus. In 1921, Campbell said than an endophytic fungus occupies a large part of inner tissue of gametophyte of *Botrychium obliquum,* but in older gametophyte it does not invade tissues about reproductive organs. The fungus fills lumen of cells but nucleus remains intact. The mycothallus of *B. lunaria* is described by Bruchmann (1906), infection taking place usually through rhizoids although it may occur directly through surface of the prothallus. Outer cells are at first invaded but later the fungus leaves them and is confined to middle and basal cells which have large nuclei and fungal clots. Starch is present only in meristem and in cells about reproductive organs while in those cells which have no starch the hyphae are filled with oil and protein. The advantage of mutualistic life of fungus and prothallus seems to consist in a holding and storing of reserve, especially oil, which is of value during summer heat and winter cold in protecting the prothallus from drying. The endophyte is present in every prothallus, living in all the inner and radially formed outer portion.

Stokey (1942) found no infection of gametophyte of *Marattia* or of *Macroglossum* grown on sterilized peat, growth being vigorous and "normal". The structure of *Helminthostachys zeylanica* prothalli is essentially similar to that of *Ophioglossum* (Lang, *l.c.*) The cells containing vesicles seem healthy but starch is usually absent from them. The fungus is healthy until growth of the sexual portion of the prothallus commences, whereupon the fungus dies and the prothallus develops up to the extent of the amount of reserve material. In prothalli of the fern *Cheiropleuria,* Nakai (1933) found fungal hyphae which entered by way of brown rhizoids and filled the median part of the prothallus where they branched and coiled to form a nutritive layer. The median layer is stimulated by presence of fungus to form 5-10 layers of cells. Uninfected prothalli were also observed and Nakai thought these may have been sterile or male.

Mycothalli in Lycopod Gametophytes:—Following Treub's discovery of endophytism in a Javan lycopod, Bruchmann (1885) described fungal structures in prothallus of *Lycopodium;* and in 1898 said that a *Pythium* sort of a fungus occurs in palisade and cortical layers of *L. clavatum* and *L. annotinum,* and is also found in rhizoids

from whence it comes in contact with the soil. Goebel (1887) stated that the lower, non-meristematic, portion of the prothallus of *L. inundatum* was always without exception inhabited by a fungus which forms coils within the cell content without killing the cell, the nucleus remaining plainly visible. From the coil a branch may go through to the next cell. The fungus is limited to one or two cell layers forming a zone separated from the exterior by several cell layers, and it is unable to penetrate the meristem, or lobes of the prothallus. The infected cells do not contain starch but drops of oil.

According to Holloway (1920), a fungal symbiont occurs in epiphytic prothalli of the New Zealand *Lycopodium Billardieri, L. B.*

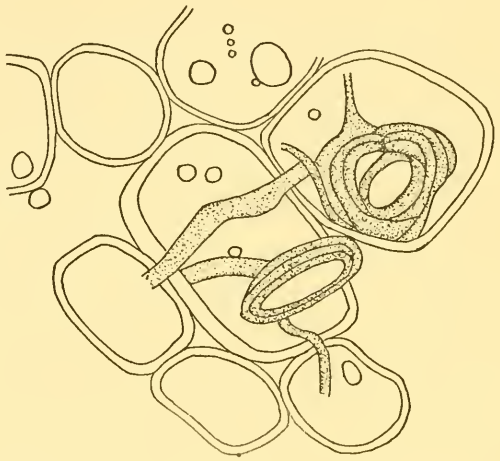


Fig. 6.—Portion of mycothallus of *Lycopodium obscurum* shown in section, with pelotons or fungal coils and oil globules (*Redrawn from* F. L. Barrows, *in* Contr. Boyce Thompson Inst. 7:299, fig. 34, 1935).

gracile, L. varium, and in the epigeic species *L. cernuum, L. laterale* and *L. ramulosum.* In the epiphytic species, the fungus occupies the base of the prothallus even in epidermal cells at the prothallial point, and grows forward with the prothallus, occupying a zone between the epidermis and the central conducting cells. Fungal coils soon disappear in many of the cells, their place being taken by clusters of darkly staining oval "spores" which are probably used by the prothalli; but oil droplets were not seen. In the epigeic species, fungus is present only in the epidermis of prothallus but occurs in inner tissues of protocorm where only "spores" were observed.

An endophytic fungus, thought to be an Ascomycete, is described from prothalli of *L. lucidulum* and *L. obscurum* var. *dendroideum* by Spessard (1922). It enters through rhizoids or through epidermal cells (or, the author suggests that the fungus may be leaving the plant through these structures) and spreads to within 2-3 cell layers of meristem. The mycelium is coiled in lower part of prothallus (the fourth layer from outside) and does not enter the palisade. Spore-like bodies were found in *L. lucidulum,* sometimes with chromatin-like content and sometimes with fine hyphae proceeding from pores as though the body were germinating; while in some cells were found true spore-bodies. Stokey & Starr (1924) cite fungal infection of *L. complanatum, L. obscurum* and *L. clavatum;* and state that fungal hyphae were usually found in great abundance in the soil in which prothalli were growing. But in culture Barrows (1936) found that an endophyte isolated from a *Lycopodium* did not aid development of germinating spores of *L. complanatum* var. *flabelliforme;* in fact, it proved impossible to grow the plantlets at all beyond a ten-cell stage. In nature, Barrows (1935) found endophytic fungi in gametophytes of *Lycopodium* sporophytes, including *L. annotinum, L. clavatum, L. complanatum* var. *flabelliforme, L. lucidulum, L. obscurum,* and *L. tristachyum.*

Mycorrhizomes in Ferns:—Originator of the term "mycorrhizome" was Dangeard (1891), who described such a structure from several species of *Tmesipteris,* although not very clearly. He described and figured an apparent Hartig net but said the infection seemed typically endotrophic; and he figured what appear to be vesicles. But mycorrhizomes have long existed: Andrews describes a fossil mycorrhizome from a coal-ball; and doubtless there are innumerable fern mycorrhizomes were there eyes to see. A few descriptions of them come from particularly close observers, such as van Tieghem (1870) who found mycelial hyphae of a parasite (*sic*) coiled about dark masses in large cells of the inner zone of *Osmunda regalis* and several other ferns. Rayner (1927) cited and figured infection of *Pteridium.* Lohman (1927) showed vesicles in his illustration of *Adiantum pedatum* and figured an apparent digestion stage for *Botrichium.* In general, however, ferns remain for future investigation, because they are not, like the pines, an economic group that commands attention.

Mycorrhizomes in Orchids:—Aside from studies of ferns and fern allies, the only other rhizomes to be studied for endophytes are those of orchids, except for the following: Kamienski (1881) de-

scribed infection in rootstocks of *Monotropa;* Oliver (1890), in *Sarcodes;* MacDougal (1900), in *Pterospora;* and Pfeiffer (1914), in *Thismia.* An endophyte was discovered in an orchid rhizome by Link in 1840; while an early student of orchid mycorrhizomes was Prillieux (1856), who took his historical introduction back to Tragus of 1552, confirmed Schacht that threads penetrating tubers of *Neottia* are fungal hyphae; and stated that at St. Germain he had found sand grains agglutinated in a mass about the orchid. Within the rhizome the 2-3 outermost cortical cell layers were filled with a yellowish-brown material (a material which he found in a great many genera) and the cells containing this material retain their nuclei. These nuclei became very large and often had two nucleoli. The cells having the brown matter regularly contained filaments wound without order about the central mass in the cell; and not infrequently the filaments branched and penetrated through the cell-wall into another cell. After a time this matter diminished in amount, from which fact it may be inferred (said Prillieux) that it serves for nutrition of the plant. Recall that this description was written 29 years before publication of Frank's epochal paper.

Pfeiffer (1877) confirmed this report for *Neottia,* finding fungal infection constant and supposing that the fungus takes the place of root hairs which the orchid lacks. A most detailed study of this same orchid was made by Werner Magnus (1900): he found 3-4 of the outermost cortical layers of cells infected,—sometimes even 6 layers. In this paper Magnus distinguished between "host" and "digestion" cells. In the host-cell the fungus never degenerates: "The cells here pictured, which always possess that ring of thick-walled hyphae with various modifications and the coil of fine median hyphae,—these cells in which the fungus does not degenerate but remains living to the last, shall be designated henceforth as host-cells (Pilzwirthzellen)" (p. 216). Thick-walled hyphae run ring-formed, in various modifications out to the cell-wall and send out fine, thin-walled haustorial hyphae which gain control of the whole cell,—haustoria which seem fitted for passage of nutrient. These cortical ring-like hyphae remain alive after death of the root.

In contrast to the host-cell region, in the digestive region the fungus always degenerates. Thin-walled, protoplasm-rich hyphae grow through the cells in thick coils but very soon die; or after they have formed protein (as Eiweisshyphen) their content is taken up by the cell and the residue is pressed together while at the same place or at a place mostly lying in the middle of the cell a clotting formation takes place, which results in their separation with a portion of the plant plasm as a clot, which is a dead unchangeable waste product con-

sisting of plant and fungal material. Of the fungus-inhabited layers, the digestion cells take the outer and inner while the host-cells take the middle. The digestion cells are defined by MAGNUS as follows: "If, in *Neottia,* the fungus in a cell does not take on the modification which characterizes the host-cell but branches again after the 'meristem condition' into thin-walled hyphae that inevitably encounter a certain developmental process,—death, robbery of content and final mantling into a clot, a development not less sharply delimited than in the host-cell,—we shall designate such a cell a 'digestion-cell' (Verdauungszelle)" (p. 223). Infested cortical cells are enlarged, and later formed cells are also enlarged, causing a change in the whole structure. The plasm continually surrounds the fungus in the digestion cell and upon death of the fungus a copious formation of vacuoles takes place. Vacuoles neighbouring the wall-layer which is free from the fungus unite to form a large sap-vacuole and thereby separate the clot, which either remains suspended in the sap-vacuole or is completely separated from the protoplast by formation of a new internally lying plasm-layer. Plasm of the fungus-inhabited cell never dies before death of the whole root. Plasm segregated in the clot becomes changed into a cellulose sort of a substance. Upon migration of the fungus there arises a fine-grained starch which soon dwindles but after death of the fungus reappears in a modified form. The nucleus becomes constricted or amoeboid and intensely chromatophilic, but after phagocytosis is completed the nuclei return to their former barrel-shape.

BERNARD (1899) described the mycorrhizome of *Neottia* as exhibiting three zones of cells: (*1*) a starch layer; (*2*) several layers of cells filled with intertwined mycelial filaments; (*3*) epidermis, without starch or hyphae. *Spiranthes autumnalis* differs from other *Neottiae* (according to BEAU, 1913) in being annual, but it has mycorrhizomes which are the organs of reserve and at time of flowering of the orchid are invaded by an endophytic mycelium as evidenced by a pronounced yellow colour given the sections through bodies resulting from digestion of mycelial coils. Towards the end of the flowering season new "roots" are formed which must be infected from the soil.

Orchids other than *Neottiae* have received attention: *Calypso* has a coralloid-branched mycorrhizome but LUNDSTRÖM (1889) failed to find infection in plants of *C. borealis* collected in southern Sweden. But in *C. bulbosa,* MACDOUGAL (1899) found fungus living in outer cortex but not passing out through nodal trichomes; its hyphae are septate and form vesicles. Inner cortex and apex are free from infection. *Corallorhiza arizonica,* according to the same author, has the coralloid rhizome represented by papillae which are infested early by

a fungus which fills the mediocortex and grows forward with the apex. The nucleus is seldom affected by hyphal invasion. *Corallorhiza innata* of the Alps has a coralloid rhizome which bears papillae from which tufts of hair arise, and at the tips of the hairs chemical changes seem to take place. Hyphae pass directly from the soil through the hair into the rhizome, going through the outermost layer of cells (which are rich in starch) to a zone in which they coil within thin-walled cells. There is a paucity of starch in this zone but within is a third zone in which starch increases in quantity as hyphae become less numerous, and all stages in degeneration of fungus may be seen in cells of this region. The nucleus enlarges and contains bodies which stain deeply with Hoffman's blue (Jennings & Hanna, 1898).

In the Australian orchid, *Rhizanthella,* Pittman (1929) described fungal infection of the rootless rhizomes to a depth of ten cell layers, the epidermis being fungus-free. Clots are illustrated as chiefly in the exocortex. Infection was through hairs borne on the mycorrhizome. No arbuscles, vesicles or sporangioles were seen, but the hyphal clots degenerate into a golden-brown mass.

In *Gastrodia* the fungus inhabits superficial cells of the fleshy succulent rhizome (McLuckie, 1923), the fungus being *Armillaria* (Kusano, 1911).

Various other orchid mycorrhizomes have been described, as in *Orchis, Cephalanthera,* etc. The general structure is always the same however, as summarized by Burgeff (1909): The mycorrhizal fungus enters through hairs into the most external cells of the mycorrhizome and penetrates the cortex even to the endodermis, dissolving whatever starch is present as it goes. Then the hypha coils within the cell, and the cell plasm digests it, the undigested remainder being surrounded by a membrane that excludes it from the living portion. A few hyphae, in many species of orchid, grow out of the rhizome again and form spores. In German orchids, Ad. Fuchs (1924) found digestion occurring chiefly from autumn into winter. Penetration of the fungus is accompanied by solution of the starch in the plant cells. The fungus follows the concentration gradient and only in such places as react to the fungal passage. The so-called protein hyphae contain an evident preponderance of glycogen, and the designation of protein in connection with them is an error. As a result of living in a region poor in N and P, the orchid undergoes modification (Fuchs & Ziegenspeck, 1925): There is an early cessation of root-development; the rhizome swells and takes over the root function as the roots dwindle and disappear. There is lessening of the water intake, a crumpling of the habdrome while the leptome is kept open.

In *Thismia americana,* Pfeiffer (1914) found underground struc-

tures which appeared like rhizomes with secondary branches, inhabited by an endophyte just beneath the epidermis; and there were finer hyphae internally in the cortex.

Mycocaryopses and Infection of Aerial Organs:—Since this book is devoted to endophytic roots and infected rooting structures, it is not deemed advisable to enter into a discussion of endophytic infection of other structures. Yet it is established that mycotrophy exists in such plants as *Lolium,* a grass in which the fungus lives symbiotically with the immature sporophyte. The "seeds" of *Lolium,* which are strictly fruit and seed together and technically known as "caryopses", harbour a fungus which is said to have been discovered by Vogl in 1897. Described by Guerin, by Hanausek, and by Nestler, in 1898 and by Hiltner in 1899, and by well-nigh a dozen investigators since, the fact of endophytic infection of *L. tementulum* is well established. Not only does it occur in recent specimens of this grass but Lindau in 1904 described similar infection from grains recovered from an Egyptian tomb about 4,000 years old. But other grasses, according to Marchal (1902), lack such infection.

A detailed study of *Lolium* was made by McLennan (1920), who described intracellular infection in the aleurone layer, hyphae penetrating also the scutellum wherever the two were in contact. Fungus is present in embryo sac at or immediately after fertilisation, and the ovum is infected before any divisions have taken place in it. Hyphae sometimes extend from base of ovary into staminal filaments where they become peculiarly knotted. In development of the embryo it is seen that endosperm is formed by an "endospermic cambium", and "if the fungus does not keep pace with the absorbing power of the endosperm, no hyphal layer is formed in the ripe grain, but hyphae can then be found in the scutellum and embryo". Endospermic cambium persists as the aleurone layer, which receives a supply of nutrient from the fungal system. McLennan concludes that "the association of the fungus with *Lolium tementulum* and *L. perenne* is probably a well-marked case of symbiosis, comparable in many respects with that met with in *Calluna vulgaris*". She also says: *L. perenne* is unable to fix nitrogen in the total absence of external supplies of combined nitrogen."

Rayner (1915) had described a constant infection of seed-coats of *Calluna* but stated that the embryo is never infected, a mycorrhizal infection resulting by infection of the plantlet from the seed-coats. Rayner asserted that the fungus grows through aerial organs of *Calluna* but Freisleben (1934) decided that infection in this plant

is not as general as thought by Rayner. Lewis (1924) described stem infection of two other ericads and also of *Picea* and *Larix*. Barrows (1941) found the endophyte of *Epigaea* widely distributed in stem, flower, pollen, young ovules and on ripened fruit and seed; and Bose (1943) reports a similar condition for Casuarina in India, infection of seedlings occurring from the seed-coat.

In *Lolium* there is also infection of aerial portions. Neill (1940) described an endophyte in the leaves, while Freeman (1904) said that all organs of the plant except the pollen may be infected.

LECTURE VIII

MYCODOMATIA

Significance of the Term:—Literally, the word "mycodomatium" means "fungus-chamber". FRANK (1891) said that on the basis of nutritional physiology, endotrophic mycorrhizae and root nodules may be considered together: their morphological differences will be taken care of if we call one "endotrophic mycorrhizae" while nodules of alder and legumes are called "Pilzkammer" or mycodomatia. But as nodules of legumes contain bacteria rather than fungi, we prefer to limit the term mycodomatia to those hypertrophied structures found on *Alnus* and a number of other plants which are caused in whole or in part by fungi. In using the word "mycodomatium" in the essential sense given it by FRANK, we realize that we are not following the original meaning as used by LUNDSTRÖM in 1887. MELIN (1936) has revived the term in its proper sense and applied it to all mycotrophic structures, those in which the symbionts "in einem Verhältnis gegenseitiger Förderung stehen". Perhaps we should follow MELIN's lead, but we encounter two difficulties: (*1*) It is very uncertain that all—or any—of the mycotrophic symbioses are true mutualisms, and it would be extremely hard to sort them out in classes of mutualist and non-mutualist. (*2*) If we gave up the word "mycodomatium", we would have no term to apply to those hypertrophied structures known as nodules, excrescenses, tubers, tubercles, etc. The term "mycocecidium" has been applied to them, but this term refers to galls and is generally understood to refer to a parasitic structure. Perhaps FRANK's "Pilzkammer" should be the term used!*

All leguminous nodules caused by bacteria are ruled out of our study. Known from the days of DUHAMEL DUMONCEAU in the middle of the 18th century, nodules of legumes have attracted much attention and their nutritional processes are of related interest to those of mycodomatia. It is true that endophytic infection of leguminous roots is widespread but vesicular-arbuscular mycorrhizae are not nodules.

In the present state of our knowledge it is impossible to state positively the exact character of mycodomatia; but it is evident that—regardless of how they are formed—they are outgrowths of a larger sort than mycorrhizae. The latter are swollen side-branches ("short-

*BAAS BECKING has called attention to the mistake of making a false analogy between symbiosis in leguminous nodules and the symbioses occurring in leaf-nodules and mycorrhizae (*cf.* Naturwet. Tijdschr. v. Nederl. Ind. 102:120, 1946).

roots") while mycodomatia are often enlargements of the larger ("mother") root, either of the root as a whole or in part, or of a subterranean stem. Smaller excrescences are termed tubers, tubercles, bulbs, etc., although the actual differences between them are not great unless we consider the former as primarily lateral outgrowths. As to the exact nature of nodules, there is obvious disagreement, but perhaps it will be found that they are chiefly the result of a bacterial stimulus and the fungi which are often if not always associated may be associate commensals, if such an expression may be used. Again, the exact nature of tubers, etc., is not altogether clear, but it seems that they are ordinarily called forth by a fungal stimulus, although other influences acting on the cell-plasm may equally well produce the tuber. Of course a tuber is strictly a stem (as in tuber of potato) and bears scale-leaves; but the word is also freely applied to tuberous roots as in the dahlia, and in both cases the enlarged growth appears due to a fungus, hence the justice of the term, mycodomatium. In orchids, the mycodomatia are sometimes true tubers, as in *Aplectrum* or *Tipularia,* or are tubercles or enlarged roots. Bulbs and corms are apparently also to be classed as mycodomatia.

Psilotum:—Our information on *Psilotum* infection is meagre, but it is certain that Gallaud (1905) listed this plant in "Series 4" with the orchids, and said it was similar in its mycotrophy with *Tamus*. *Psilotum* is an "humus saprophyte" in which rootage organs are much branched rhizomes that bear small gemmae on the subterranean shoots. Yet *Psilotum* can grow asymbiotically as discovered by Bernard and reaffirmed by Costantin (1925, 1936), who said that the plant had been grown asymbiotically at the Museum at Paris for 132 years.

Cycads:—The root tubercles of *Cycas,* according to Bottomley (1907), are morphologically lateral roots showing a central vascular cylinder with a well-marked endodermis completely surrounded by "bacteroid" tissue. These tubercles are dichotomous and perennial, and they differ from leguminous nodules, which are of limited growth. Kellerman in 1910 isolated N-fixing bacteria from *Cycas* nodules, while Life (1901) found in *C. revoluta* both bacteria and hyphae of a fungus which resembled *Rhizobium.* These organisms, he said, are confined to the mediocortex. Life thought the functions of the tubercles were aeration and N-assimilation but decided it is difficult to speak with certainty of the symbiotic relations of the various organisms within. Spratt (1915) said that the tubercles are formed primarily by *Bacillus radicicola,* and noted that in them four symbionts are concerned,—two bacteria, an alga and the cycad.

Podocarpus:—Nodules on *Podocarpus chinensis* were noted as early as 1893 by KELLNER, and were described in 1896 by v.TUBEUF. They were examined in detail by NOBBE & HILTNER (1898), who found a fungus growing throughout the root, and forming nodules from within the roots, hence they concluded that the nodules or mycodomatia are true endotrophic mycorrhizae. Plants were grown in pure quartz sand for 5 years and watered with non-nitrogenous culture solution, the plants growing luxuriantly and presumably securing N from the air through the mycodomatia. Later, HILTNER (1903) said that N-fixation was shown for *Podocarpus* but not with the same certainty as with *Alnus*. KONDO (1931) also wrote of N-relations of these plants but his work is hidden in the Japanese language.

Processes occurring within the *Podocarpus* mycodomatium were described by SHIBATA (1902), who found a large hyphomycete which by branching filled the whole cell: the host nucleus increased in volume and assumed amoeboid form, dividing amitotically until as many as 8 nuclei are formed which become distributed in the cell, then becoming amoeboid once more. When the fungus has attained its full growth it is digested by the host-cell and the nuclei may then resume their normal condition and divide mitotically. A proteolytic enzyme capable of digesting fibrin was demonstrated in the tubercle. SHIBATA corroborated MAGNUS and FRANK, that the fungus is subservient to the host-cell. HILTNER (1903) said that the host-cell digests and absorbs not only the plasm but the chitinous wall of the fungus; and he also noted nuclear activity in these cells. "Bläsen" in *Podocarpus*, he said, are equivalent to JANSE's sporangioles, being partly formed and then digested. MCLUCKIE (1923) described nodules of *P. spinulosa* and *P. elata* as of dual character. He said that these species, like other species of the *Podocarpineae*, are actively engaged in N-fixation by virtue of bacteria present in cortical cells. The nodules are modified lateral roots and arise from the pericycle, their normal growth being checked before they emerge from the cortex of the main root. Root-hairs, he said, are commonly present. Nodules and cortex of main root frequently contain fungal hyphae and peculiar spore-like bodies belonging to the fungus; the surface of the nodule and main root is frequently invested with a loose tangle of fungal hyphae, some of which enter the root tissues. The nodules of *P. chinensis* and *P. nubigena* are occupied by a fungus (SCHAEDE, 1943) which is considered a harmless parasite since it has so slight a connection with the soil; but the arbuscular structure is markedly developed.

Casuarina:—Nodules filled with a gummy mass were found by JANSE (1897) on *C. muricata* in Java. KAMERLING (1911) described

Casuarina nodules from coral islands in the bay of Batavia, and said that in section it is seen the nodules possess a small central strand while larger or smaller groups of cortical cells are filled with protein-reacting bodies resembling leguminous bacteroids. KAMERLING supposed that the nodules were responsible for N-fixation, and the same function was ascribed to them by ADINARAYANA (1924), MOWRY (1933), and NARASHIMHAN (1918), the last isolating bacteria that fixed N. MIEHE (1918) tacitly inferred the same function, and asserted that these nodules are mycodomatia, the symbiont being a small hyphal fungus which heavily infests the cortex, passing directly from cell to cell but never invading the vascular bundles.

Myrica:—BRUNCHORST in 1887 had mentioned tubercles in *M. Gale* which were described by BOTTOMLEY (1912) as modified lateral roots. Three branches arise from the end of each primary nodule and afterwards the stele grows out through the apex of the nodule into a hair-like root. In each mature nodule four regions may be recognized, *viz.:* apical meristem, "infection thread" area, "bacterial zone" which includes most of the cortex, and basal zone devoid of bacteria but with the cells containing oil drops. At maturity the bacteria disappear and basal zone encroaches until it finally replaces all the others. In old nodules, filling a majority of cortical cells and sometimes the base of young nodules, mycorrhizal fungi are found. Fungal hyphae, said BOTTOMLEY, were earlier thought to be responsible for nodule formation and it is possible that they may be of mycorrhizal nature and of benefit to the *Myrica* plant. BOTTOMLEY caused nodules to develop by inoculation; he also showed fixation of free N. SCHAEDE (1938) considered the causal organism of this *Myrica* to be *Actinomyces* and he gives a well illustrated account of the infection.

In *M. rubra,* SHIBATA (1902) found the "fungus" (which he believed to be *Actinomyces*) confined to a definite "ring" in the cortex. In *M. carolinensis,* the author found beneath a cuticularized epidermis a cortex of 10-11 layers of rounded cells, larger internally and full of protoplasmic content. A zone of 2-3 layers commencing about the fourth from the outside of cortex is a "bacterial layer" containing comparatively large "rods" which are densely clustered and deeply stained. About the outside of the domatium there is more or less a weft of branched septate and geniculate hyphae, dark in colour. It is difficult to demonstrate infection but neverheless in the outer cortex there is the appearance of intracellular hyphae; while many of the cells have content suggestive of partially digested protoplasm which takes a reddish stain while the bacteria stain blue. The latter divide transversely to form rosettes. Under oil immersion, strands can be

seen connecting adjacent "masses" through cell-walls, tangentially. The cell nucleus is not hypertrophied. Rosette formation by *Actinomyces* is mentioned by YOUNGKEN in a study published in 1915 on the *Myricaceae;* and he said that this organism later penetrates tracheae and grows out into the seed. In "*M. cerifera,*" HARSHBERGER (1903) described mycodomatia inhabited by *Frankia.* He supposed that these structures were intermediate between ectotrophic mycorrhizae as in *Monotropa* and endotrophic forms as in *Thismia.*

Alnus:—In alders occur the earliest described root excrescences which may be termed mycodomatia. MEYEN in 1829 gave the first description of tubercles in alder (so far as we are aware), and considered them as "pseudomorphosed roots" in the ends of which there is a parasitic growth comparable to that of *Lathrea,* etc. MEYEN was sure that he had shown them to be "ganz vollkommene parasitische Gebilde" and that they were formed by "gleich anderen vollkommenen Organismen". Since then there have been numerous other descriptions given but their exact nature and symbionts still remain unsettled. Apparently alder nodules are not caused by one organism nor do they always have the same physiology, for some investigators describe them as bacterial, others as fungal. Thus CERNIK (1937) lists the "fungi" of alder nodules as *Frankia, Schinzia,* and *Actinomyces,* all of which are presumably bacterial; while PIESCHEL (1929) cites *Lactarius lilacinus* and *L. cyathula* as always associated with alder and presumably the mycodomatial symbionts, with *Gyrodon rubescens* a probable third symbiont. Two investigators report synthetic mycodomatia for alder,—PLOTHO (1941) and ROBERG (1938). ROBERG grew seedlings of four species of alder in a synthetic nutrient solution with a suspension of ground root-nodules isolated from each of the species. Only healthy seedlings reacted to inoculation by nodule production; and in all cases the symbiont was *Actinomyces alni.* Because of the frequent presence of this organism, SHIBATA (1902) termed the alder mycodomatia cases of vegetable actinomycoses.

A number of papers describe nutritional processes of alder mycodomatia. SHIBATA, already mentioned, tells of the "bläschen" or small bodies formed by the "fungus" in mycodomatial cells and their subsequent digestion; he also described clots which contained, besides some fungal hyphae, a number of little rounded drop-like or oval structures which he termed "sekretkörper". But ZACH (1908) did not find these bodies in *A. glutinosa* (SHIBATA worked with *A. japonica*), but considered the broken threads or "Stäbchen" of SHIBATA as concentrated cell-content of the hyphae while spore-like knots and bacteria-like threads are degenerate forms of hyphae which, he

claims, absorb much water and fill the entire cell lumen. Terminal swellings of the hyphae are also degeneration stages of the fungus which are ultimately digested by the host-cell, during which process the fungal masses pass through various degenerative stages. Spherical, oval and other shaped bodies of an oily consistency appear during the digestive process and to these he applied the name "Exkretkörper". Shibata had described a proteolytic enzyme from alder mycodomatia. The author has seen numerous yellow clots in outer cortical cells of *Alnus rugosa,* which alder has coralloid mycorrhizae in addition to mycodomatia. Klečka & Vukolov describe fungal digestion in alder and other nodules and regard the fungus as provider of starch and protein. Hiltner (1896) claimed that alder nodules assimilate free nitrogen; and he also found that $CaCO_3$ stops their growth. Borm (1931) said that in *Alnus* it has been found possible to prove that the bacteria fix N, but that it is not certain the nodules formed only by fungi can perform this process.

Polygonum:—Ectotrophic mycorrhizae are constant in *P. viviparum,* not only in the countless adventive roots but in the bulblets (Hesselman, 1900), which must then be considered as mycodomatia.

Raphanus:—Molliard (1920) stated that radish produces tubers perfectly well under sterile conditions when supplied with sugar and CO_2 in sufficient quantity. The presumption is that in nature radishes are "fungus-chambers" called forth by infection.

Tribulus:—In sandy places of the Gov. Cherson in Russia, among dry arid sand vegetation, Issatschenko (1913) found fleshy green specimens of *T. terrestris* that bore nodules on their roots,—small white ones on thin roots and larger dark nodules that recalled leguminous nodules. In section, dark septate hyphae were evident, clothing outside of the nodule and penetrating into it in places while within, the hyphae were thinner and lighter in colour, and proceeded from cell to cell. Disappearance of starch from the nodules was observed. Issatschenko thought that these mycodomatia were true mycorrhizae and agreed with Bernard that using of the starch increases osmosis of the cell and with it the water intake.

Legumes:—In addition to bacterial nodules and endotrophic mycorrhizae, legumes possess mycodomatia. Janse (1897) described fungal nodules in *Pithecolobium montanum,* a member of the *Mimosaceae:* the cortex contains 2 layers of tannin cells separated by 2 layers of parenchyma and in the latter the fungus is found, but it never

enters tannin cells. The nodules recall those of *Casuarina.* Fungal invasion of *Orobus* was discovered by Frank (1879), and he figured arbuscles and vesicular swellings in the nodules of *O. vernus* and *O. tuberosus;* and Frank considered that the hyphae were transformed into "Sproszellchen".

Ailanthus:—In the Erlangen Botanic Garden, Andreae (1894) found that sturdy side-roots of *Ailanthus* had irregular tuberous outgrowths of 5-40 mm. diameter placed directly on the root cylinder and composed of smaller bodies in a grape-like cluster. Their structure was thought due to the higher plant and not induced by the fungi (mostly Pyrenomycetes) found in the nodules. Further studies are awaited on these structures.

Ceanothus:—Nodules were noted on *C. americanus* by Beal in 1890 and were described by Atkinson (1892). While resembling in form leguminous nodules, he found the causal organism was a "fungus" which he named *Frankia ceanothi.* Material was collected from Alabama and Michigan, and similar nodules were found on *Alnus serrulata.* In a more extended study, Arzberger (1910) said that infection is through epidermis or root-hair and the mycodomatium consists of 3 systems of tissues: an outer corky layer, a middle cortical tissue, and an inner vascular bundle. In the cortical layer are infested, hypertrophied cells, the nuclei being enlarged. He noted also three stages in development of the fungus,—mycelial, "sporange" and digested. No "Exkretkörper", as described by Zach, were found, but an enzyme capable of digesting fibrin was found. He said that symbiosis exists but both host-cell and fungus dies. A very different description was given by Bottomley (1915), who considered the nodules purely bacterial, formed by bacteria of the *Bacillus radicicola* group. As no nodules were formed on *C. americanus* in England, he imported material from America, securing nodules also of *C. velutinus.* The bacteria, when isolated, grew in pure culture and fixed N.

Elaeagnus:—The same discrepancies must be noted in descriptions of *Elaeagnus* nodules. Brunchorst and Frank agreed at first for their fungal nature; then Frank (1887) withdrew to the position that the nodules are merely reserve organs, containing no symbiont. Zach (1908) described them as fungal and similar to those of *Alnus;* Nobbe (1892) believed that he had demonstrated N-fixation with them. On the other hand, Spratt (1912) identified the causal organism as *Pseudomonas radicicola,* but stated that it does fix free N. The author found coralloid mycorrhizae but no nodules on *Shepherdia argentea* in northern Minnesota.

Hippophaë:—In 1887 Brunchorst spoke of the "well-known" nodules of *Hippophaë* and considered their possible action in fixation of free N. These nodules were rediscovered by Arcularius (1928) who gives a detailed description of their structure and ascribes their formation to a fungus. There is no infection of the vegetative apex nor is starch present in cells infested by the fungus, though abundant elsewhere. The fungal hyphae swell near the cell-nucleus and form "little heads" which gradually swell with a fine, deeply-staining material. This material is then apparently digested and finally disappears, with coincident nuclear changes. The relation of fungus to host is not obligate, and the author supposed that soil must be inoculated in order to have mycodomatia produced. But Borm (1931) ascribes the nodules to bacteria which, he said, multiply in an enormous way until they fill the whole cell (the nucleus remaining intact) and then digestion occurs.

Coriaria:—In *C. japonica,* Katakoa (1930) ascribed N-fixation to the nodules, saying that plants with nodules make vigorous growth while without them growth is retarded. Shibata (1917) said that the endophyte, a typical *Actinomycete,* forms a rich weft separate from the cortical tissue, the colonies of which in the host-cells have consecutive partitions with centripetal pectinate hyphae arranged club-wise about the vacuoles which are filled with cell-sap. The root-nodules of *Coriaria* in respect to anatomical differences surpass all others and its characteristic symbiosis-tissue is quite similar to that of legumes.

Eucalyptus:—According to Dufrénoy (1922), swellings are found on axes of young *Eucalyptus* plants, of which the origin is unknown.

Daucus:—Sterile achenes of carrot were germinated in a mineral gelatine with some addition of glucose (5.0-7.5 parts per 100) and plants were grown in large tubes plugged with cotton; but with glucose there was poor growth. In sterile humus soil with addition of mineral salts, in tubes plugged with rubber stoppers, the air had 5 parts per 100 of CO_2, and the plants grew well, in 40 days forming a tuber 1 cm. in diameter (Molliard, 1920).

Ericads:—von Tubeuf (1903) said that on the Chiemsee Moors at Bernau in Bavaria, the largest of the Vaccineae is *V. uliginosum,* which has its root system deeply sunk in sphagnum. If whole stocks are drawn out of the sphagnum, a portion of the attached roots is obtained with a tender root-work; while on thin rootlets are found

copious nodules of various sizes and forms which appear as little clubs although they may have a very delicate continuation as a thin rootlet. In section they are seen to have a central cylinder with pronounced water tissue and peripherally a normal cortical tissue. von Tubeuf thought that neither fungi nor bacteria are responsible for these structures. He found them on all woody plants of the moor except pine, that is, on six ericads including *Calluna* and *Andromeda.*

But for *Arbutus,* true mycodomatia caused by a fungus are described by Rivett (1924) and by Dufrenoy (1917). Inoculated rootlets developed into small pear-shaped tubercles, said Dufrenoy, on which nearly all the epidermal cells develop into root-hairs, around which algae and bacteria collect and form a mucous. The fungus invades external layers of cortex which stores large quantities of reserve material as "tannin" while medullary tract and rays are crowded with starch grains. Rivett, in describing the endophyte in old tubercles, said that infection by fungus keeps pace with production of new cells by growing point, and digestion and reinfection proceed successively. Thus peripheral cells, except at growing points, are to be found filled with partially digested hyphae. Digestion proceeds all the time that tubercles are growing and even in winter it is hard to find clearly defined hyphae. In a great majority of the cells cavities are filled with a granular mass of deeply staining material in midst of which persists a large nucleus. Endodermal sheath becomes densely filled with reserve, and the conducting tissue itself becomes blocked with deeply staining material. Tubercles persist in this condition throughout winter and early spring.

Pyrola rotundifolia possesses tubers formed by inordinate radial increase in size of epidermal cells as result of fungal infection. At first the hyphae are intercellular but later they penetrate the cells and fill them; the nuclei become hypertrophied and then disappear. A mantle is finally formed about the root (Kramář, 1899).

Solanum:—Tuber formation of the potato, according to Bernard's early work (1901) is called forth by an endophytic fungus, *Fusarium solani* (later called *Rhizoctonia solani*). In pure culture with the fungus, tubers were freely produced while in soil that was little infested tubers were sparse. He said that, according to statements made by de l'Ecluse in 1601, when potato seed was first planted in Europe, flowering but not tuber-forming plants were produced, so that to secure a crop of tubers, older tubers rather than seed had to be planted. Today, plants grown from seed produce tubers the first year because with general cultivation of the potato the fungus is widely distributed in the soil. Bernard noted still further (1902*a*)

that tuber formation is not dependent on the fungus *per se* but to a certain sap concentration, for cuttings of potato plants placed in aqueous sugar solutions produced tubers. This discovery he attributed to MARCHAL. A critical concentration exists for each plant; and BERNARD thought that tuberisation in all cases depends directly upon a certain degree of concentration of cell sap. But ordinarily the habitual provider of this sap concentration is a fungal parasite (1902*b*), which produces the optimum concentration for diastasic ferments. BERNARD grew the fungus, *Fusarium solani,* in a maceration of potato sap and found it increased the sap concentration as indicated by a lowering of the freezing point.

That tuber formation is connected with sap phenomena was indicated further by the work of ROLFS (1901), who found that small tubers were formed on the stem when a stricture was placed about the stem, either by the fungus or by artificial girdling, and the sap was prevented from flowing to the region of tuber formation. It may be noted incidentally that presence of glucose in concentrations of 1/100 to 1/10 mol. is termed essential to cell division and elongation in wheat roots (BURSTRÖM, 1941). But MOLLIARD (1915) found that even when the plant is placed in a sugar solution there was no tuber formation until gaseous interchange (which increased sugar absorption) was suppressed. MAGROU finds that normal tuberisation may be obtained glucose (also glycerine), in combination with action of light (Ann. d. Sci. Nat., Bot., XI, 5:135-136, 1944).

Believing that tuber formation is always induced by fungi, BERNARD (1911) investigated other plants and found that *S. Dulcamara* and *S. Maglia* (the latter from Chile) also contained an endophyte. JANSE (1897) had found the same for *S. verbascifolium* in Java. MAGROU (1914) found furthermore that the endophyte of *S. Dulcamara* could induce tuber-formation in *S. tuberosum,* hence there is no necessarily specific endophyte; and this observation was confirmed by COSTANTIN (1935). Yet under cultivation the endophytic fungus is lost and the potato plant produces tubers without it (MAGROU, 1921; CASTAN, 1941); and the suggestion is made that dunging destroys the fungus,— which still lives in the wild form, *S. Maglia* of Chile. With further study it was concluded that tuber formation in the potato is an "acquired habit" of the plant in cold climates, the climate having the same sort of action in tuber formation as the fungus; for the potato in cold climates, either in high latitudes or in high altitudes, produces tubers normally whereas in warm climates this power is lost (COSTANTIN, 1922). Potatoes grown at 1400 m. produces more tubers than those at 560 m. (COSTANTIN, 1935*a*), a result confirming LEBARD & MAGROU (1935), who found that there is an altitude where the yield

is maximum. Miège (1936) found that refrigeration for not longer than 4-5 months restored vitality to the potato quite as well as a change in altitude. Loss of the endophyte in this fashion explains Jumelle's (1905) problem of why the isolated *Fusarium* seemed to have little importance in tuberisation of *S. tuberosum* and *S. Commersonii.* Indeed, Castan (1941) concluded that a symbiotic fungus is not necessary to tuberisation, at least at low altitudes.

These statements were modified somewhat by Costantin (1936). Thus, while tubers of cultivated potato do not contain symbiotic fungi, certain varieties contain mycorrhizal fungi just as the wild forms. Furthermore, while infestation is usually abundant, it may be sparse or completely lacking in certain individuals. Again, in late summer at high altitudes, small ("microscopic") tubers were formed in conjunction with symbiotic fungi that, left in the soil, spontaneously reproduce the plant; but at lower altitudes the symbiotic fungi are lacking and the tubers perish during the winter. This action was confirmed by Joseph (1935), who notes also that "microscopic" tubers differ in colour.

Melampyrum:—*M. pratense* utilizes the humus of the moss, or grass, tussocks in which it lives through delicate protuberances or absorptive organs produced from the roots. These protuberances were found actually growing into dead objects (Koch, 1887).

Orobanche:—Henfrey (1849) suggested that the whole tuberous base of the plant is concerned in absorption, just as in orchids. Further studies of mycotrophy in this plant are awaited.

Composites:—Molliard (1920) has been mentioned already for his work on radish and carrot: under similar sterile conditions he was able to induce tuber-formation in *Dahlia,* that is, under optimum conditions of sugar and CO_2 supply. Swollen adventive roots were formed within 6 weeks, and Molliard concluded that "under favourable conditions" micro-organisms are not necessary to tuber formation,—although it is not explained how plants in nature are to secure flasks, sugar solutions and rubber stoppers as substitutes for the aforementioned endophytes. An actinomycosis is described by Dufrenoy (1920) for *Adenostyles,* but he does not state definitely that there is an enlargement of the tissues.

Juncus:—Tubers on *Juncus* were mentioned by Chatin in 1856, also by Cameron in 1886, who found root-swellings likewise on *Ruppia maritima, R. rostella* and *Eriophorum vaginatum.* Weber

(1884) made a detailed study of various *Juncus* plantlets and found them inhabited by a fungus and swollen into tubers the size of which depends on activity of the rush. The fungus is present only in radially enlarged cells of the periblem where it forms coils of reagent-resisting hyphae, and surrounds the nucleus; or hyphae penetrate to other cells. As in rust fungi, the hyphae are surrounded by a cellulose layer that is continuous with the membrane of the penetrated cell. Spores are formed by the fungus, which assume a barrel-shape and become surrounded with a thick dark wall. In winter, the mass of the former tuber in wet earth is full of ochre-yellow spores which germinate naturally in February. The fungus is considered to be *Entorrhiza cypericola,* placed in the *Tilletiaceae.* Grütter said that in *J. Tenageia* the fungus encloses tip of root, penetrates epidermis and forms special structures in it. The stele is much reduced. Lagerheim (1888) described *E. digitata* from *J. articulatus* in Switzerland. The roots were deformed into root galls and contained an abundance of yellow spores, and the fungus was extracted with difficulty. In the Black Forest, *J. articulatus* bore mycodomatia in very sandy and not too wet soil but they were absent from moor and loam soils, occurring in the uppermost soil horizon.

Molinia:—This grass forms a "molinetum" on sterile sands of northern Germany and elsewhere, its rhizomes and interlaced roots acting as sand-binders. It overwinters as swollen basal nodes while the roots are endotrophic, never ectotrophic. A line drawing indicates fungal coils in inner cortical cells and a possible "sporangium", perhaps a vesicle. Data are presented on N content of tuberous rhizomes and seeds. Plants were grown for three months in culture solution and sand, and one plant at the end of the experiment was found with fungus-free root system, while its rhizome-base was filled with starch. Hence grasses must be examined in considerable numbers to determine true extent of infection for some, like *Molinia,* may be facultatively mycotrophic (von Tubeuf, 1903).

Cyperus:—Magnus (1879) described a fungus, *Schinzia cypericola,* living in roots of *C. flavescens.* Through its activity the root swells into a simple tuber or, if the roots branch, into a branched tuberous body. In *Schoenus ferrugineus* there are mycodomatia containing normal fungal hyphae (Renner, 1935).

Asparagus:—Root nodules of *Asparagus* have been described in Japanese by Fujita (1940).

Allium:—In an extended study of *A. roseum,* CAPELLETTI (1931) isolated a fungal endophyte which he referred to *Rhizoctonia.*

Orchids:—Tuber formation of orchids was described by FABRE in 1855 but interest in its significance dates from BERNARD (1902). The latter found, as in potato, the causal fungus is *Fusarium* (*Rhizoctonia*), and that tuber formation takes place very early in development of the plant; yet the tuber itself, at least the parenchymatous interior, remains fungus-free. BEAU (1914) also said that in the adult plant, tuberisation may take place without fungal invasion. BERNARD found a retardation of development in the orchid plant which goes hand in hand with nodule formation and storing of food-stuff, and to him it seemed the result of a sort of poisoning caused by the endophyte. So long as the plant is free from infection there is active growth of leaves, flowers and fruits; but tubers are formed only after entrance of fungus. Later (1909), BERNARD classified orchids as facultatively mycotrophic (as in epiphytic members) and constant mycotrophs. According to BURGEFF (1910), the fungus is found in roots and protocorms of almost all orchid plants. GALLAUD (1905) classified orchid tubers in Series Four, and stated that the endophyte is intracellular and produces coils (pelotons) which sometimes remain inactive (host-cells) or are digested (digestion cells). A curious condition was described by BARSALI (1921) in which two horizontal tubers are formed in addition to the ordinary ones, the former tubers supposedly making use of humus of the top-soil.

LECTURE IX

STRUCTURE OF MYCORRHIZAE

The Kinds of Mycorrhizae:—With insight which characterized his work, FRANK early stated that there are two principal sorts of mycorrhizae, the coralloid sort found with forest trees, and the endotrophic which he illustrated from ericads. That general distinction into basidio- and phycomycete types is found to hold generally good. Yet it must be remembered that mycorrhizae are formed primarily by the higher symbiont and that their form is determined by the vascular plant producing it: the fungus is of secondary significance. One fungus or another, or several fungi together, may invade the root, but the mycorrhizal form will be essentially the same in all cases: its form is characteristic for the higher symbiont rather than for the fungus. This fact is emphasized by WOODROOF (1933) who says: "... it is seen that the influence of the fungus in the gross morphology of mycorrhizal roots is slight. The presence of the fungus is to all outward appearances merely incidental."

Mycorrhizal Compared with Non-Mycorrhizal Roots:—Not all the rootlets of a mycorrhizal plant are necessarily mycorrhizal, and a distinction must be made in the case of woody plants between *long-roots* or "roots of extension" that grow rapidly through the soil, and *short-roots* or small laterals which serve principally for intake of materials from the soil. WOODROOF (*l.c.*) calls attention to the fact that not all short-roots are infected and that short-roots are not started by the endophyte but, being formed, are invaded. The structure, whether invaded or not, is the same in both cases as regards gross morphology. Long-roots are considered to be fungus-free, and when short-roots are likewise uninfected they usually bear root-hairs; but if infected by symbiotic fungi, the short-roots become shortened and swollen in their development. Yet HATCH (1937) presents some evidence to indicate that mycorrhizal fungi stimulate their growth and thereby increase the absorbing surface areas. The rootlet that bears mycorrhizae is called a *mother-root* (following NOELLE, 1910): the mother-root may renew its apical growth and extend out into the soil as a *pioneer-root*. To illustrate, a simple mycorrhiza is a mother-root bearing a very few elongate laterals; a coralloid mycor-

rhiza is a mother-root plus small coral-branched short-roots. But in the case of annual plants or biennials, any of the secondary roots may be infected, or even the adventitious roots; while in several plants with aerial roots, these are turned into mycorrhizae.

External Form:—The form of a mycorrhiza is characteristic for each species of plant and generally the form is constant for a genus and even for a family. Thus, all cupulifers have a coralloid sort of mycorrhiza; *Juglans* have simple, and *Acer* have necklace-beaded mycorrhizae. Yet it must be noted that MELIN (1925) states that mycorrhizal form is greatly influenced by the sort of salts present in the soil. The least complex form is the *simple* mycorrhiza which consists of elongate monopodial rootlets such as occur in *Liriodendron, Cornus* and *Fraxinus*. As described by MELIN from pine heaths, simple mycorrhizae may grow to 10 mm. length and 0.2 mm. diameter, and are ordinarily without root-hairs. Apparent root-hairs on simple mycorrhizae may on inspection turn out to be fungal setae, which often simulate epidermal outgrowths.

The *coralloid* mycorrhiza is said by ULBRICH (1924) to have been first described by HARTIG in 1851. It is branched freely like coral, the "mother-root" bearing numerous short branches that, in comparison with the simple mycorrhiza, may grow to 1 mm. or more length and 0.4 mm. diameter; *i.e.,* they are short and thick. They are well seen in pines, oaks, birches, and in the German are called "Gabelmykorrhizen", a shrub-like sort of structure. The *racemose* mycorrhiza, as found in spruce and other forest trees, is formed by lateral rootlets branching monopodially in two rows upon a main axis. When coral-branches cluster thickly at one place to form a sort of "witch's broom", the cluster is called a *rhizothamnion;* or, in the German, a "Büschel". Rhizothamnia are seen on pine (MÜLLER, 1902) and oak, and are said to be characteristic for Casuarinas (MIEHE, 1918). Or, the cluster of short dense branches formed by dichotomy may be weft about with mycelium to form a nodulous lump called a *tuberous* mycorrhiza, or in the German, a "Knollenmykorhiza". It is not truly a nodule, neither a tuber; and is said to have been first observed by MÜLLER on *Pinus montana.*

Pearl-necklace mycorrhizae are formed in yet a different way. They commence as ordinary racemose mycorrhizae or perhaps as widely spaced coral-branches but through intermittent growth successive additions are made and a constriction is left between each two additions. Thus are developed the "pearl-necklace" beads so characteristic of *Acer,* and found in various other plants. Such mycorrhizae may be found on *Pinus virginiana* when the latter grows in droughty

soil, and doubtless in most if not all cases this sort of mycorrhiza is associated with intermittent growth.

Pseudomycorrhizae:—Infection of a short-root by a fungus does not necessarily result in formation of a mycorrhiza, for there are many cases in which the infecting fungus is a parasite. Such "false mycorrhizae" had long been observed but were named "pseudomycorrhizae" by MELIN (1917), who observed them on pine and spruce growing in Swedish moors. The pseudomycorrhiza is thinner and simple, or sometimes monopodially branched, in pine; the hyphae are intracellular and penetrate even the meristem, and must be considered parasitic. MELIN thought that MÖLLER's "ectotrophic mycorrhiza" was the same as a pseudomycorrhiza. LATHAM, DOAK & WRIGHT (1939) said that under field conditions most non-mycorrhizal short-roots of pine become pseudomycorrhizae, thus reducing the absorbing surface of the roots and their ability to take up mineral nutrients.

Pseudomycorrhizae are thin and lack the basal constriction that marks the mycorrhiza; then, too, mycorrhizae are usually lighter in colour than the mother root, at least when young, whereas the pseudomycorrhiza is dark in colour.

The Colours of Mycorrhizae:—In earlier days some attention was paid to colours of mycorrhizae: Thus, MANGIN (1910) cites *Quercus* with white and rose-coloured ones, *Fagus* with yellow and blue. MCDOUGALL (1914) presented a classification of mycorrhizae based in part on colour, *viz.:* bright yellow, brown, white. MASUI (1926) said there are three types of ectotrophic mycorrhizae on roots of *Alnus firma* var. *Sieboldiana,*—white, yellow and dark. A yellow colour of the root is characteristic of mycorrhizae of potato (MAGROU, BOUGET & SEGRETAIN, 1943).

Two things influence mycorrhizal colour, *viz.* age, and the fungal symbiont. In general, young mycorrhizae are light in colour, often a pure glistening white; and they become darker as they grow older until they usually turn brown, although very old mycorrhizae may be black. But a black pine mycorrhiza may split its sheath and produce a white tip of renewed growth under favourable conditions. Or, a black colour may be given the mycorrhiza by a fungus long known but more recently described as *Mycelium radicis nigrostrigosum,* which usually develops strands of hyphae from the surface. Other fungi may cause other colours, as yellow, reddish or pale violet; but as the mycorrhiza grows older these colours tend to disappear.

The Exterior Surface:—Phycomycete and simple mycorrhizae are usually smooth of surface and lack visible mycelial coating, while coralloid mycorrhizae are often shaggy with hyphae. In the latter, when the hyphae are densely interwoven they form a *mantle* that is weft as closely as a tissue and when young may have a white satiny surface, but when older becomes "fuzzy" with free hyphal ends. Peyronel (1922) termed this mantle a *micoclena,* or it would perhaps be better written "mycoclena", Greek for fungus-mantle; while Ziegenspeck (1929) called it a *mycoderm,* when it is a pseudotissue. In both the smooth and the shaggy mycorrhizae there are doubtless numerous hyphae that extend into the soil, passing from the soil certain materials into the interior of the root. Such hyphae have been appropriately called *Communication-hyphae.* Being delicate, they are inevitably broken in removing the mycorrhiza from the soil and could be observed directly, if at all, only by some "glass-plate" method. Communication-hyphae are the "root-hairs" of a mycorrhiza.

But oftentimes the fungus produces short setose hyphae that project evenly from surface of the mycorrhiza and simulate root-hairs,—except that of course root-hairs are not developed from a mycorrhiza, neither are they formed on a root-cap! Yet Gordon (1936) describes and figures "root-hairs" over tip of a mycorrhizal short-root. Presence of setae over apex of the rootlet or mycorrhiza is indicative of their hyphal origin, and close examination with high-power stereoscopic binocular microscope shows continuation of the seta with a close-weft mantle hypha. Woodroof (1933) describes pecan mycorrhizae with three sorts of setae on the surface,—flask-shaped with long necks, with short necks, or stellate with intermixed spines. Mangin (1910) also described and figured setae, as hairs dilated at the base and tapered regularly to a point,—length 100-150 μ, diameter 5-6 μ at base.

Or, hyphae may coalesce on or near the surface of the mycorrhiza to form strands known as *rhizomorphs* which are similar to, but usually smaller than, the rhizomorphs found in soil or under bark of dead trees.

It is quite possible that root-hairs and setose hyphae are present simultaneously on a root, and fungal infection may be through root-hairs; or it may occur directly through the epidermal wall. Root-hairs are not developed, usually, to any extent on a plant provided with mycorrhizae; yet their presence depends to a considerable extent upon the soil in which the plant is growing, for in a forest the roots will be mostly turned into mycorrhizae whereas in cultivated soil root-hairs are more to be expected. Acid humus is not favourable to growth of root-hairs, and when such are formed in so unfavourable

an environment they are apt to be crumpled, shortened and otherwise indicative of an untoward environment. These considerations again show the casual nature of the mycorrhizal relationship.

The Mycorrhizal Apex:—The effect of habitat on rootlet is perhaps never better shown than in the root-cap region. In aquatic roots where there is little resistance to apical growth, the apex may be freely exposed; or, if there is a root-cap as in *Eichhornia,* it grows to

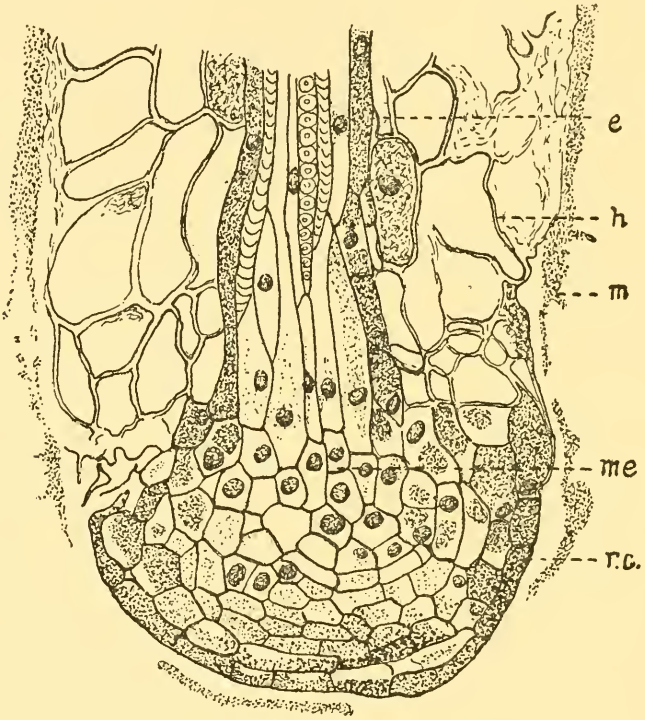


Fig. 7.—Longitudinal section through apex of a mycorrhiza of *Pinus rigida.* r.c., root-cap; me, meristem; m, mantle; h, Hartig net; e, endodermis, filled with "tannin".

a relatively enormous size and fits loosely over the apex. *Aesculus,* grown in water culture, produces roots that "keine Wurzelhaube haben und diese ihnen von ihrer ersten Entwicklung an fehlt". (Klein & Szabó, 1880). In ordinary root-hair roots (as in *Zea*), there is a close-fitting root-cap formed from a definite zone of proliferation in the proximal portion of the apical meristem. Pressure of rootlet

against soil causes the cap to fit closely while friction of growth causes sloughing of external cells. But contrast with the mycorrhizal apex is decided, for in the mycorrhiza the root-cap is small, densely filled with content and is closely covered over by a mantle of firmly weft hyphae. At first white or light-coloured, it becomes dark with age and the apical meristem finally ceases activity. In healthy mycorrhizae, said MANGIN (1898), the root-cap instead of being lost is conserved although invaded by mycelial filaments; and in part the cells are detached from each other. The only change caused by pressure of the mantle is a regular hemispheric form given to the root-cap. The next year he pointed out that the mycorrhizal root-cap is never exfoliated but persists throughout the life of the mycorrhiza between the mycelial mantle and the more or less hypertrophied external cortical cells. And, as already noted, the apex may be covered over with "root-hairs", as described by GORDON (1936) for broad-leaved trees, by MÜLLER (1886) for beech, and by ZACH (1909) for *Sempervivum.*

The mycorrhizal apex in short-root of Corsican pine is neatly illustrated by ALDRICH-BLAKE in Oxford Forestry Memoirs: Details of meristem, cap, and infected tissues are well-shown and are conformable to those of other species of pine. But in 1874 it was supposed (by JANCZEWSKI) that gymnospermous roots have no cap nor true epidermis although the primary cortex is exceedingly voluminous over the root-apex, replacing the cap. MACDOUGAL (1900) found the root-cap little developed in *Monotropa* but many-layered in *Sarcodes* and *Pterospora:* but in all cases the tip is covered with mycelial mantle that crushes the cap-cells.

Under favourable conditions of growth, as during a rainy season that succeeds a drought, the apical meristem may renew its activity and again cut off cells. As a result of pressure thus set up, mantle covering the apex is split, exposing a new mantle that has developed beneath, and the mycorrhizal short-root continues its growth in length.

Renewed Growth:—This is such a common phenomenon it is strange that it is not more generally remarked. MASUI (1926) devoted a paper to the subject, finding that "the mantle-clad root-apex of the completed mycorrhiza in *Abies firma* (Form A and Form B), *Abies Mayriana, Alnus japonica* and *Pinus densiflora* can renew its growth breaking through the mantle." The mantle splits in various directions, hyphae grow out from the split and later cover the exposed root-tip. Or, the quiescent meristem renews its growth after bark and mantle have been sloughed off.

Normal renewal of growth in mycorrhizae of *Taxus* was described and figured by Prat (1926): "Mamelons" formed during one season are quiescent over winter but "Au printemps, les cellules du méristème central se remettent à proliférer, formant un massif qui traverse les assises externes comme fait une radicelle pour les tissus de la racine-mère. A l'intérieur de ce massif apparaît un nouvel endoderme qui se raccorde à l'ancien. L'écorce du nouveau segment de racine est donc séparée de l'ancienne écorce par des assises de tissus

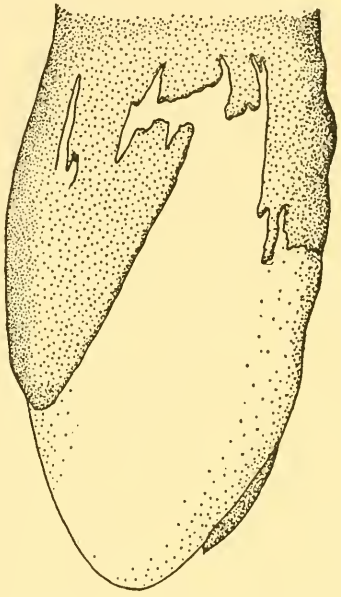


Fig. 8.—Renewed growth of a mycorrhiza of *Pinus virginiana*, showing splitting of mycoderm and extension of root-tip (Collected near Baltimore, 22 February 1930).

morts et pigmentés." This is the method of formation of "pearl-necklace" beaded mycorrhizae.

Ectotrophic vs. Endotrophic:—Mycorrhizae have been conventionally classified as ecto- and endotrophic, the distinction originating with Frank (1887) who said: "We (may) designate all those forms as 'ectotrophic' which have the nourishing fungus external to itself, and as 'endotrophic' where it (the fungus) penetrates into the interior of certain root-cells." But with passing years doubt was

expressed whether any such absolute distinction could be made between the two. KAMIENSKI (according to GROSGLIK, 1885) had early said that in cupulifers hyphae penetrate into inner tissues of the root and extract nutrient; and MELIN (1922) said that intracellular infection does occur in ectotrophic mycorrhizae of *Larix,* suggesting that earlier the intracellular hyphae may have been overlooked (MELIN, 1923*a*). MELIN called these mycorrhizae which combined characters of both the preceding sorts, *ectendotrophic.* Intracellular hyphae in ectotrophic mycorrhizae were described by MASUI (1926*a, b*) for *Alnus* and *Abies;* by JOHANSEN (1931) for the cactus, *Neomammillaria,* which has a white mantle about the root with hyphae penetrating between epidermal cells and into cortical cells where apparently vesicles were formed; and by KLEČKA & VUKOLOV (1935), who found individual hyphae enter cells as short branches or barrel-shaped structures.

But ENDRIGKEIT (1937), in a study of both sorts, came to the conclusion that in ectotrophic mycorrhizae of *Tilia, Quercus* and *Pinus* in East Prussia there is no such infection: "The opinion recently expressed as to the endophytic character of the ectotrophic forest tree mycorrhiza finds no support in the writer's investigations". He thought that no nutritional or physiological significance can be attributed to the occasional observation of rudimentary intracellular infection or the common intensification of the "Hartig net" on a decayed primary cortex. In pine, YOUNG (1938) observed that "Mycorrhizal development varies from strictly ectotrophic in the case of untreated controls, through the typical ectendotrophic in the 1½ lb. of S treatment to the almost purely endotrophic in the 3 lb. S treatment." Both ecto- and endotrophic mycorrhizae were found on Corsican pine by ALDRICH-BLAKE (1930).

Ectotrophic Mycorrhizae:—In cupulifers, according to FRANK (1885), the root is surrounded by a fungal mantle that is mostly many-layered; now colourless, now a light to dark brown pseudoparenchyma which lies close upon the true epidermis. It sends hyphae in between the cells but never quite into the innermost layers of root cortex, growing always in the membrane of the cell only which they thickly weave about; but they never enter the cell lumen. The outer surface of the mycorrhiza is not infrequently smooth but root hairs are never formed, their place being taken by a felt of loose hyphae that extend out into the surrounding soil.

MANGIN (1910) described the ectotrophic mycorrhiza of *Castanea* as follows: The diameter of the root augments and the piliferous cells, covered over by mycelial mantle, are never permitted to form

hairs; they elongate in an oblique direction at an angle of 45° with the axis of the root and their length attains the double or the triple of their diameter. He notes that, whereas in cupulifers only the epidermis is involved, in beech, etc., two layers of cells are hypertrophied; while in pine, fir, larch, a great number of layers are affected. In any case, hyphae intrude between walls of the cells,—dissolving out the cementing pectate and forming "palmettes" which may cover

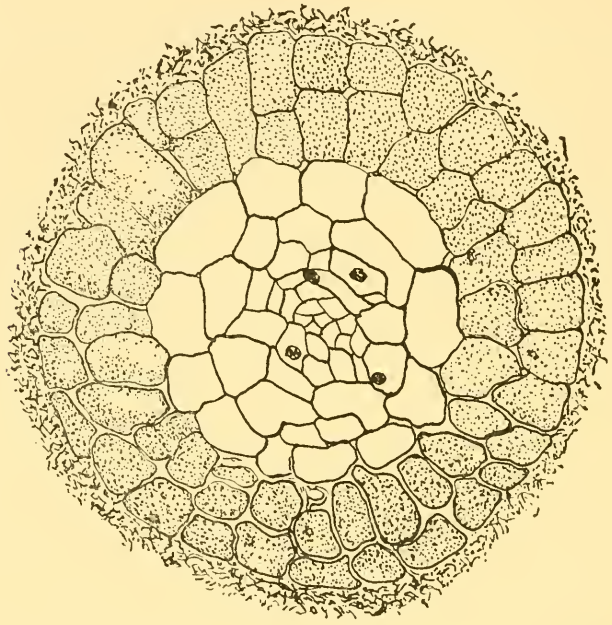


Fig. 9.—Cross-section of an ectotrophic mycorrhiza of *Quercus montana*, indicating mantle or mycoclena and cortical cells filled with endophyte. (*Original drawing from* a slide prepared by Dr. K. D. Doak).

the radial face of the cell. MacDougal & Dufrenoy (1944) state that in pine the middle lamella of the outer cortex is traversed. Seen in section the intruded hyphae between the cortical cells appear as a netted structure which has been called the "Hartig net" after Th. Hartig who early observed it, although he supposed the structure was composed of anastamosing canals.

Besides in cupulifers, ectotrophic mycorrhizae occur in ericads and some other plants. Hesselman described such structures from the arctic *Salix herbacea* and in the herbaceous *Dryas octopetala* and

Polygonum viviparum. According to CHRISTOPH (1921), these mycorrhizae occur in ericads only in such species as *Arctostaphylos Uva-ursi* which form coralloid mycorrhizae in humus, the fungal mantle living at the expense of epidermal cells. Again, he says that such mycorrhizae occur only in such species as have subterranean organs differentiable into rhizome and root, and especially when, in a humus-rich location, a copious branching of short-roots has been produced.

Ectotrophic mycorrhizae are, accordingly, distinguished by an exterior mantling of hyphae that have been called a "mycoclena" or, when tightly weft into a pseudotissue, a "mycoderm"; from which extend hyphae into the surrounding soil. The endophyte penetrates intercellularly into the epidermis, or in some sorts of plants into the cortex, in the latter case forming the structure which appears as the Hartig net. If intracellular infection occurs, then the mycorrhiza is not strictly ectotrophic. These mycorrhizae occur on roots of woody plants, rarely on herbs.

Endotrophic Mycorrhizae:—It is evident that a diverse series of mycorrhizae have been grouped under the term "endotrophic". FRANK (1887) included here those of ericads, which have a mantle and approach the ectotrophic condition; mycodomatia have been included also; and GALLAUD (1905) included mycothalli. Endotrophic mycorrhizae are typically developed in all *Pinaceae* except the *Abietineae* (which are ectrotrophic) (*cf.* NOELLE, 1910); in orchids, and in herbs in general.

GALLAUD (1905) distinguished endotrophic mycorrhizae into "series", *viz.* (*1*) *Series of Arum maculatum:* Mycelium at first intracellular in the protective layer of the root, then intercellular and lodged in the plasm; arbuscles and sporangioles generally simple and without very precise localization. Examples are cited from monocotyls, dicotyls, and *Angiopteris.* (*2*) *Series of Paris quadrifolia:* Mycelium always intracellular, arbuscles or sporangioles generally composite, not terminal and harboured in a definite region of the root. Certain angiosperms are cited as examples; also *Araucaria, Podocarpus, Sequoia* and *Ophioglossum.* (*4*) Omitting the hepatics, which are his third series, the *Series of orchids:* Mycelium always intracellular, taking the form of coils (pelotons) which sometimes remain inactive (host-cells), sometimes are digested (digestion-cells). Examples: orchids, *Psilotum* and *Tamus.*

In other words, in various endotrophic mycorrhizae there may be simple hyphal coils formed as in the orchids, or arbuscles and sporangioles are developed,—the nature of which will be detailed shortly. Or, it might be said that there are basidial endotrophic mycorrhizae

(orchids), and phycomycetous endotrophs as in the vesicular-arbuscular sorts. Since the terms "vesicle", "arbuscle" and "sporangiole" have been adopted from the French, there seems no valid reason why the term "peloton" should not also be adopted, to describe the endotrophic mycorrhizae of orchids and some other plants.

Peloton Mycorrhizae:—In *Neottia Nidus-avis,* according to W. Magnus (1900), the root-inhabiting fungi possess very few and irregular connections with the outside. The 3-4 outermost layers

Fig. 10.—Cross-section of endotrophic mycorrhiza of *Acer Negundo.* Invading hyphae are coiled in the cortex.

of cells beneath the epidermis are completely and without exception inhabited by the fungus while in rhizome and stem even six layers may be infested. Infected cortical cells are enlarged and later formed cells are also enlarged, causing a change in the whole structure. Within the cortex two sorts of cells were distinguished, "Pilzwirthzellen" and "Verdauungszelle". According to Magnus, after the hyphae had formed protein (Eiweisshyphen) their content was taken up by the cell and the residue was pressed together, while at the same place or at a place mostly lying in the middle of the cell there begins a local clotting formation. They then become separated, with a portion of

the plant plasm, as a clot (Klumpe) which is dead, unchangeable waste product. On death of the fungus a copious formation of vacuoles takes place and by union of vacuoles a large sap-vacuole is formed in which the clot remains suspended. A new cell membrane may be formed about this body: Thus are formed the clots, the "gelbliche Stoffe", which puzzled earlier observers.

Burgeff (1909) observed formation of hyphal coils external to the mycorrhiza: The hyphae excrete a drop of water into which an hyphal branch grows, and this branch, hindered by the outer surface tension from growing out of the drop, is consequently rolled into a spiral inside. Thus might form the coils (pelotons) of the mycorrhiza.

Three sorts of orchid mycorrhizae were distinguished by Burgeff (*l.c.*), *viz.* (*1*) Neottid (including most sorts); (*2*) Coralloid (*Corallorhiza* and *Epipogon*); (*3*) Sporangiole (including a tropical genus only). Later (1931) he also described *vesicle* formation which, however, is not common in orchids.

Hypertrophy of the nucleus occurs during fungal digestion (*cf.* Magnus, Arcularius).

The peloton mycorrhizae, therefore, are characterized by intracellular infection whereby hyphae coil within the cells to form "pelotons" which are afterwards to be digested: vesicles and sporangioles are rarely developed in this sort.

Vesicular-Arbuscular Mycorrhizae:—The "phycomycete mycorrhiza" is distinguished by possession of arbuscles and vesicles. Neill (1944) records as a further distinctive feature a constant anastamosis of the hyphae which sometimes results in formation of a closed system of intercommunicating passages traversed by moving protoplasm.

Vesicles were first recorded by Mollberg in 1884 (*cf.* Groom, 1894) but were described by Janse (1897): "I give the name of 'vesicles' to the spherical or ovoid swellings which occupy extremities of the hyphae. In the young state these organs contain only a small amount of protoplasm and their cavity is occupied almost entirely by large vacuoles. Little by little the quantity of protoplasm augments, nutritive reserves accumulate and finally they are filled with a granular mass in which are mixed oily droplets. I have found analogous bodies in a great number of plants. May they be compared to the cysts which are present in other fungi, and a role in the asexual multiplication of the endophyte be attributed to them? I incline to that belief." It may be noted that vesicular swellings were figured by Frank (1879) in nodules of *Orobus* while they were described still earlier (1847) by Reissek for certain monocotyls and for *Cochlearia*.

Vesicles may be inter- or intracellular, or even formed outside the root. Gallaud (1905) regarded the vesicles as "organe de réserve souvent temporaire" (an opinion which Demeter, 1923, shared); and he described their formation as almost always terminal: the hypha ceases to grow in length and swells at the extremity while a very dense protoplasm accumulates there and the nucleus multiplies rapidly by division. Groom (1894) thought that the vesicles merely appeared terminal but actually were intercalary, a tip growing into another cell and there forming another or several more vesicles. Similarly, Pyke (1935) said that in *Cacao* vesicles form in the epidermis and from there a stout hypha grows into cortical cells adjacent; but Laycock & Dale (1945) state that vesicles were never seen in mycorrhizal roots of Cacao, and arbuscles but rarely; and they would prefer not to use the term "vesicular-arbuscular" to describe these mycorrhizae. In *Vinca,* the vesicles form either terminally or intercalary; and in age, in addition to a number of nuclei they have great fat vacuoles with protoplasm between them like cross-walls.

Vesicles were described in detail by Peyronel (1923), who considered them to be reproductive bodies of the endophyte; and he figured spores within them,—a view that was held by a number of earlier authors (*cf.* Rives, 1923); but "most authors consider them cysts which survive death of the organ and reinfect the new root." Bernard (1911) figured "germination of a vesicle" isolated and placed in a hanging drop; while Johansen (1931) described and figured "spores" (vesicles?) which, he stated, germinate and renew the infection, in certain cacti. In *Stapelia variegata,* Busich (1913) figured a germinating vesicle. Butler (1939) attempted to grow the fungi from external mycelium and vesicles and, though fine hyaline new hyphae were sometimes obtained, they did not grow extensively. The vesicles of *Vitis* were figured as thick-walled and containing four rounded bodies like vacuoles (Rives, 1923); while in *Gossypium* they are terminal, round, oval, or irregular (Sabet, 1939). In *Lolium* (McLennan, 1926), vesicles are oval (65×45 μ), usually terminal but sometimes intercalary and mostly intercellular. At maturity they have thickened walls but later lose their content and collapse. They appear to be "an attempt towards spore formation", while for the higher plant they exist as a "temporary reserve organ". At an early stage they are packed with fat which is later given up to the host plant.

No vesicles were formed in synthetic mycorrhizae although such occurred in nature (Bouwens, 1937).

Vesicles are also formed by the endophyte of *Conocephalus,* both in nature and in culture (Beauverie, 1902; Bergamaschi, 1932):

they are large and spherical in *Monoclea* (Cavers, 1903). They occur in older prothalli of *Ophioglossum* (Bruchmann, 1904; Campbell, 1908) while in prothalli of *Botrychium* thin-walled vesicles are often so abundant as to fill the cells with a botryose mass (Jeffrey, 1898). Baas-Becking (1923) said of *Botrychium:* "The so-called vesicles occur, thin-walled, apparently osmotic products, filling sometimes the whole cell; or two vesicles may occur in a cell."

Arbuscles:—These are said to be even more constant than vesicles but more delicate and difficult to observe. It was the privilege of Gallaud (1905) to describe these organs which had escaped earlier observers. He said that a branch of an intercellular hypha enters the cell-wall and within the cell gives off 3-4 new branches and then dichotomises until it forms what looks like a floccose mass. These ramifications enter into the host's protoplasm. "A cause de leur forme générale rappelant en petit celle d'un arbre très chevelu j'appelerai *arbuscles* ces formations singulières très importantes sur lesquelles j'aurai à revenir plus longuement." An arbuscle terminates the extension of each hypha. Gallaud thought that arbuscles are absorbing organs (organe absorbant ou suçoir). They are simple or composite (arbuscles composés) when a complex of arbuscles, sporangioles and hyphae. Vuillemin thought that arbuscles are less a characteristic production of the endophyte and more a result of the reaction of the host-cells to invasion by a foreign body.

Digestion of arbuscles by the host results in formation of the *sporangioles* of Janse, otherwise called "prosporidi" by Petri. Janse said: "These sporangioles, as I call them, owe their embossed aspect to the relief which the spherical bodies that are contained in the interior cast under the extremely thin membrane, bodies which I designate by the name of 'spherules'. The spherules are filled with a quantity of little 'granules'. In spite of the name of sporangioles which I give to these organs, I am far from affirming that they have a part in the propagation of the endophyte. It must be stated, however, that they are found, with only two or three exceptions, among all the plants which I have studied." Gallaud said that transformation of arbuscles into sporangioles is almost always very rapid, and Janse noted in a number of the plants he investigated that the sporangioles later freed their content to form a gummy mass, or at maturity, they formed very fine granules that diffused through the cell. And Endrigkeit (1937) decided that intracellular arbuscles cannot be interpreted as assimilatory organs since they are digested as they are formed and show no indication of hyphal development from their terminal branches, but

rather as proliferations induced by the growth promoting stimulus of the cell-sap.

Ericaceous Mycorrhizae:—As described by KAMIENSKI (1884), *Monotropa's* epidermis is covered with a fungal mycelium of septate hyphae which form a compact pseudoparenchymatous mantle 2 or 3 times as thick as the epidermis. The fungus lives on the surface and never penetrates living cells, but sometimes does so in older portions where these cells are filled with a brown content. In older parts of *Monotropa* roots the epidermis disorganizes at the same time the mycelium develops. But FRANCKE (1934) said that in epidermal but never in deeper layers of cells, hypae are found entering and surrounding the nuclei, becoming filled with reserves that are emptied into the host-cells as "plasmoptyse". The reserve is now absorbed but no excretion was observed, and no significant change in nuclear condition was seen. After digestion has occurred, vesicular swellings of hyphae dwindle. Only one hypha enters an epidermal cell. Tannin is no protection from the fungus for hyphae are found in tannin cells as well as in tannin-free. MACDOUGAL (1900) said that the mycelium of *Monotropaceae* consists of an external absorbing system and an internal one which fills the epidermal cells. In the Monotropas, vesicles, sporangioids, and sporangioles fill the cells, and "probably serve as organs of interchange."

In *Arbutus* (DUFRENOY, 1917) the roots are clothed with a dense mantle of hyphae protected by a thick greyish membrane. The fungus penetrates external layers of cortex and sends haustoria towards starch grains. Late in the season (RIVETT, 1924), hyphae penetrate more deeply in the cortex, but digestion continues all the time the host is growing and the reserve storing tissues packed with content. In *Calluna* (RAYNER, 1927), in young roots the cortex consists of a single layer of large cortical cells each of which encloses a dense branch system of mycelium that is continuous with hyphae upon the external surface of the root. Intracellular hyphae are of relatively large and uniform diameter with abundant oily content.

Vaccinium is infected by hyphae passing through the cell-walls (COVILLE, 1910) and the epidermal cells are completely filled with coiled hyphae; and in *Andromeda polifolia* also the epidermal cells are infected by very fine hyphae (FRANK, 1887); while in *Vaccinium Oxycoccos* connection was found between external mycelium and intracellular hyphae. VOSS & ZIEGENSPECK (1929) found digestion stages ("clumps") in *Andromeda* and curious mamillae in the cranberry. In *Ledum* these authors found that in winter roots the fungal hyphae roll together to form a brown mass; in *Erica* there was less

infection; while in all ericads studied the youngest roots had some portions fungus-free.

The Pyrolas studied by FÜRTH (1920) had the mycelium spread over the whole length of the root but confined to the epidermal cells which were hypertrophied and gradually filled completely with hyphae that cause the death of the cell and result in development of "clumps".

Coralloid Mycorrhizae:—The various pine mycorrhizae are described with difficulty, largely because there is so little information available about them. Although so many papers have been published on pine, there are very few anatomical studies about them extant. That is especially true of recent times, for the trend at present is toward philosophical disquisitions on the nature of mycotrophy, a branch of learning that conveniently eliminates the drudgery of sectioning. Hence knowledge of pine mycorrhizal anatomy is largely confined to two European species,—*P. montana* and *P. sylvestris;* and there is almost nothing on American pines. But coralloid mycorrhizae are not confined to pines since they occur on other plants,—on how many others no one could say.

Knowledge of coralloid mycorrhizae (Gabelmykorrhizen) dates back at least to REESS (1887) although they are said to have been first described by HARTIG in 1851. MÖLLER (1902) said that they were well known in *P. sylvestris* while in 1908 he recorded them from sand of the Brandenburg Marches but absent from humus. Infection in *P. sylvestris* and *Picea Abies* (MELIN, 1921) is through root hair or epidermal cell and hyphae grow at first intracellularly in outer cortical cells where they form a pseudoparenchyma, but later form an Hartig net and mantle. Similarly, *Abies firma* mycorrhizae (MASUI, 1926*b*) have not only a mantle and Hartig net but intracellular infection. In *Larix* the fungus penetrates intracellularly into the roots (MELIN, 1923) and forms individual hyphae or knots which in time are digested, after which the fungus penetrates intercellularly and the mycorrhiza becomes more strictly ectotrophic.

In synthesis experiments with pine (MELIN, 1923), only ectendotrophic mycorrhizae were obtained.

Tuberous Mycorrhizae:—"Knollenmykorrhizen" were brought into the limelight by MÜLLER (1902) who regarded them as similar to the coral clusters of cycads and some legumes. They are formed by a dichotomy which is rare amongst roots, yet not called forth here by a fungus: "We stress the fact that the dichotomous branched tubercle is not invaded until after its dichotomy has been manifested". Both racemosely and dichotomously branched mycorrhizae occur

mixed on the same root. From the tubercle extend strands of hyphae into the soil, as KIRCHNER said (1908): "auch hier strahlen von der Pilzscheide förmliche Hyphen-Perrücken in den Boden aus, der zu einer dichten Masse verflochten wird." Ordinarily these tubercles live but one year but sometimes they are persistent and continue to branch, forming a rhizothamnion.

On *P. mugho* (LAING, 1923), conspicuous nodular bodies, often over ½ inch diameter, are found on trees not more than 12-13 years old. The nodules are sessile or stalked and frequently found detached in the soil. Branching of the rootlets is considered checked by the fungus which is purely ectotrophic and rarely penetrates cortical cells. NOELLÊ (1910) also failed to find the intracellular infection claimed by KIRCHNER (1908). In *P. sylvestris* nodules were described by LAING (1923) with endophytic infection, while MELIN (1922) also described them, stating that they are as large as a pea. Nodulous roots on *P. Cembra* were described by VON TUBEUF (1888), who quoted from REESS (1887), who seems also to have described them. Nodules of *P. montana* are mentioned by SOMERVILLE (1911). MÜLLER (1902) thought that they are of service in fixation of atmospheric nitrogen.

Outer Cortex and Passage Cells:—In plants that develop a thickened outer layer of cortex, or exodermis, in the rootlet, there are left some thin-walled cells in the layer that do not develop the wall-thickening; and these thin-walled exodermal cells form a convenient means of access to the cortex beneath for the invading hyphae. When so used by the endophyte these thin-walled cells are termed *passage-cells*. They were described by JANSE from Spadiciflorae and named "cellules de passage", and he said that they are found in all orchids. BURGEFF called them "Durchlasszellen" (DEMETER, 1923). Passage-cells were described from *Tipularia* by CLIFFORD (1899), from *Aphyllorchis* by GROOM (1894), from *Dipodium* by MCLUCKIE (1922), from *Vanilla* by CORDEMOY (1904), from *Vinca* and *Asclepias Cornuti* by DEMETER (1923), who found passage-cells also in the endodermis, from *Gentiana* by SCHIMMLER (1937); and they are figured for *Hoja carnosa* by BUSICH (1913), who considered the presence of passage-cells as an inherited character of the *Asclepidaceae* that aids mycotrophy and adapts the plants to their habitat.

Wall Tubules:—A hypha, passing through a living cell-wall, may stimulate the cell to form a growth about the hypha which has been called a *wall-tubule* or, in German, a "Röhrentüpfel". JEFFREY (1898) had observed in *Botrychium* mycothalli that a thick sheath

surrounds the hypha for 10 or more micra but only when the wall is cuticularized. Arcularius (1928) found that the host-plant surrounds the hypha with a cellulose layer; Francke (1934) reported that the haustorial hyphae are very early surrounded by cellulose; while Magnus (1900) had found the same in *Neottia,* and Shibata & Tahara (1917) had described an analogous relation. The sheath separates the living hypha from the host plasm. Kusano (1911) said that the cell-wall formed papillae where hyphae passed through, which were often branched or formed a "tubular sheath" that he thought was lignified. Burgeff (1932) said that the wall-tubules are composed of lamellate cellulose which would indicate by its structure that there is a diffusion current preventing a regular layering of the wall, and forming a teet-like structure.

Hartig Net:—Hyphae, on entering the cortex, do not always penetrate the cell-walls. In certain plants, particularly well seen in the

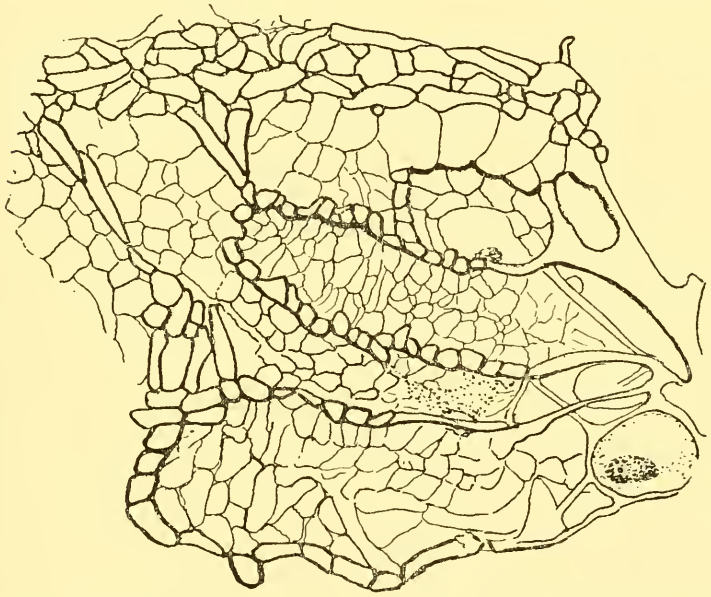


Fig. 11.—Section of a mycorrhiza of *Abies balsamea,* showing Hartig net which has formed about the cortical cells.

conifers, penetrant hyphae weave about cortical cells until they form a basket-weave structure in which the cortical cells are literally embedded. Seen in section it appears as a netted structure and is called

the *Hartig net,* after Theodor Hartig who early described it from pine. Hartig considered the delicate net to consist of anastamosing intercellular canals (Mangin, 1910:245): it had been described by Nicolai (1865) as "thickening strips", and he said that they are found not only on radial cell walls but in all places where the cells are not pressed together, so that a peculiar net is formed. He found them in conifers and the apple-tree. Reinke (1873) also described such a structure from a number of conifers, and van Tieghem & Duliot (1888), as a "réseau de soutien". In pines, Hartig-net is formed of 2-3 rows of hyphae, as in Möller's *P. sylvestris* (Shimizu, 1930); Hartig net also occurs in spruce (Melin, 1921), in *Podocarpineae* (Berggren, 1887), and in the fern *Tmesipteris* (Dangeard, 1891). Because of mutual pressure of cortical cells, hyphae penetrating between them become flattened and, branching, are formed into a digitate appressed structure which Mangin (1910) called a *palmette.* But Mangin (1898) said that there is no network in the cortex but, because of pressure exerted upon them, the hyphae are flattened into regularly branched layers between the cortical cells. Voss & Ziegenspeck (1929) said that in older plants of *Betula pubescens* the Hartig net is digested; and in pine it also breaks down (Lewton-Brain, 1901).

The Stele:—In general, mycelial infection of the central cylinder does not occur, yet exceptions have been noted. Thus, Dufrenoy (1917) found infection of all tissues in *Arbutus Unedo,* and in 1920 recorded heavy infection of pericyclic tissues of *Adenostyles albifrons.* Lewis (1924) found the endophytic fungus growing through all the tissues of all vegetative organs of *Picea* and *Larix;* and Rayner (1927:p.100) found similar growth in *Calluna* stems. Masui (1926*b*) found outer layer of pericycle in old roots invaded and in rare cases the whole central cylinder, which was subsequently destroyed. The stele of *Cupania* is also sometimes invaded (Waage, 1891). Infection of the stele was found in young roots of *Lygino-dendron* but not in older roots by Ellis (1917); while Schacht (1854) had found vascular infection of a leguminous wood from the London clay as well as in living ferns and other plants; although in these cases the infection may well have been parasitic: indeed, in all cases of vascular infection the endophyte may be parasitic.

That the endophyte influences structure of the stele is a fact not widely recognized, according to Noellê (1910), who said that "es ist bisher nicht bekannt das ein ektotrophes Myzel die Struktur nicht nur der Rindenschichten, sondern häufig auch das Zentralzylinder zu beeinflussen scheint". The "normal" (uninfected) root is diarch but

under influence of infestation becomes monarch: the phloem area becomes reduced while the xylem area is proportionately increased, suggesting an increased water intake through fungal influence. But HATCH (1937, p.131) says that in pure culture, short-roots of *Pinus Strobus* are monarch in vascular structure, and the reduction in stelar tissues is accordingly not due to invasion by the endophyte. In holosaprophytes (JOHOW, 1889), the vascular portion is always much reduced, or xylem and phloem are diversely arranged. The stele is much reduced in *Juncus Tenageia* following infection (GRÜTTER, 1886). Later researches will have to determine further the influence of endophytism on stelar anatomy.

LECTURE X

OBLIGATE SYMBIOSIS

Fungi and Trees:—That fungi are confined in their symbiosis to one species of plant was stated as early as 1841 by TULASNE, who said that *Elaphomyces granulatus* was confined to one species of tree; and GIBELLI in 1883 suggested that the mycorrhizal condition is a necessary one in the cupuliferae. In more recent years the confinement of certain fungi to the neighbourhood of certain trees has been remarked. Thus, BARSALI (1922) found that in forests about Pisa there were always the following fungi associated with the trees named: *Lactarius delicosus* under *Pinus Pinea* on sandy soils (but ROMELL, 1939, said that this fungus is rarely found with pine); *L. volemus* and *L. oedomatopus* under *P. pinaster; Boletus bovinus* under *P. pinaster* and *Juniperus macrocarpa; B. granulatus* under *P. pinaster; B. edulis* under *Quercus* and *Castanea; B. corsicianus* under *Q. ilex* (where this species grows with *Q. Suber*); *Russula grisea* and *R. emetica* together with *Lactarius* under *P. pinaster.* LANGE (1923) gives a comparable list for Denmark, and LIDL (1939) for Germany. PALM (1930) had noted a *Boletus* growing in association with *P. Merkusii* in forests of Sumatra and another bolete under *P. cubensis* in the Guatemalean highlands. YOUNG (1937) found both *Rhizopogon luteolus* and *Boletus granulatus* under the same pine tree in Queensland, and thought it possible that both are symbionts of the same tree at the same time. REESS (1885) had concluded that *Elaphomyces* is dependent for its occurrence on presence of pine. Again, HAMMARLUND (1923) noted association of *Boletus* with *Larix* in Sweden, and proved by synthesis that the fungus is mycorrhizal.

Ecological Influences:—An interesting ecological study of such occurrences was made by PEYRONEL (1917): (*1*) in *Larix decidua* woods, boleti predominate; (*2*) in cupuliferous woods agarics predominate, especially polypores; (*3*) with *Salicaceae, Populus tremula* has a rich fungal coterie while *Salix alba* has none; (*4*) *Betula alba* is accompanied by a discrete number of humus fungi, while *Alnus glutinosa* is found with *Lactarius;* but *A. viridis* lacks characteristic humus fungi; (*5*) most other ligneous species are never accompanied by humus-dwelling hymenomycetes; (*6*) meadows are characterized

by many species of *Hygrophorus* and *Agaricaceae*. In all cases the variations in the florula seem correlated with the sort of humus present.

Special Cases:—Such examples could be multiplied but the objection is raised that finding of sporophores under certain trees is not *a priori* proof that these fungi are mycorrhizal symbionts of the trees; neither does tracing of mycelial connection between the two constitute proof. But Romell (1930) offers the interesting observation that *Lactarius delicosus* not only constantly occurs with *Picea* in the region of Stockholm, but that this species disappeared upon removal of the few spruce trees that stood in a mixed pine stand. On the other hand, Dittrich (1923) said that *L. delicosus* is found in great quantities under thick-set spruce trees when the trees are young, but disappears completely after the trees have reached a certain age. An interesting case is cited by Mattirolo (1934*b*) in that *Populus canadensis* was introduced into Italy from America about 100 years ago: *Tuber Borchii* is found on this poplar and, as the fungus is recorded also from California, may have been introduced into Italy on introduced *Amentaceae*. Further, Melin (1922) said that *Boletus elegans* appeared in Sweden only after introduction of larch.

Fungi and Herbs:—Associations of fungi with herbs are likewise noted. Thus Colla (1931) found two species of fungi associated with *Dryas octopetala*. Fraser (1931) made "the first report of an obligate association of an annual herb with a mycorrhizal fungus" in two species of *Lobelia*. In a study of German species of *Polygala*, Heinricher (1900) concluded that some species at least of this genus can scarcely be considered as obligate mycorrhizal plants, for he was able to grow the plants in culture without mycorrhizae. For the orchids, Kusano (1911) thought that *Gastrodia* was unable to flower without infection as indicated by pot experiments. Obligate symbiosis is indicated by Porter (1942), who reports that *Rhizoctonia mucoroides* is probably the specific endophyte of *Zeuxine strateumatica* since this fungus is found both in Florida and in Java.

Obligatism and Nutrition:—Without multiplying examples, the argument is the same in all cases: Certain fungal fruiting bodies are found habitually in the neighbourhood of certain higher symbionts, and by careful examination a mycelial connection can be traced between the two. The objection is raised that here there is no necessary obligate relation since it is possible, perhaps, to grow the two apart in sterile culture. The fact remains, however, that the two symbionts

do grow in union, and are thus found in association. The relationship is probably not obligatory since the fungus, *e.g.*, which grows on *Pinus Taeda* in America might grow equally well on *P. sylvestris* in Europe; but in actuality the two are separated by the Atlantic ocean and have no means of coming together. In other words, the association is obligatory only in the sense that the symbionts have no other means of livelihood except in association. The humus-dwelling hymenomycetes cannot live in arid mineral soil and cannot therefore associate with dune grass or beach plum; and it may be that some are limited to certain specific compounds just as human individuals are limited in metabolism to certain polypetides. At the same time it is perfectly true that the otherwise "obligate fungi" can be grown in culture apart from their usual symbionts; yet the laboratory and the woodland are two different things, and without being too cynical we may say that our experience with natural woodlands is that they are notably deficient in supplies of Erlenmeyer flasks and culture media.*

The Ericads:—The ericads offer a much-mooted relationship. At least three cases of obligate symbiosis have been recorded for these plants: (*1*) Francke (1934), in a study of *Monotropa Hypopitys,* found that a fungus-free protocorm was never seen which, he thought, indicated an obligate mycotrophism. Kamienski in 1884 had raised the question whether the relationship were obligatory. (*2*) Paula Fürth (1920) made the unconfirmed report of obligate symbiosis in *Pyrola* in Lower Austria. (*3*) Rayner (1915) claimed to have proved an obligate relationship in *Calluna vulgaris.* A fungus identified as *Phyllophoma* was found to grow not only in roots but throughout the whole plant—stems, leaves, flower and seed-coat but not growing into the embryo. Infection of the developing plantlet was from the seed-coats; while sterile seedlings did not live long enough to develop roots. But Christoph (1921) reported that *Calluna* in nature is facultatively mycotrophic since the fungus is entirely absent from habitats lacking humus, indicating that an obligate symbiosis does not exist.

The Problem of Calluna:—*Calluna* was further studied by Knudson (1929). He used seed grown in the U.S.A., and germinated the seed on Rayner's solution A to which was added 1.5% of standardized agar. The seed was sterilized in a Ca hypochlorite filtrate, usually for 30 min., and transferred by a looped platinum wire

*Systemic infection is also claimed by Bose for *Casuarina.* He says that only the resting embryo is free. Seed infection was also found in tomato, apple and several kinds of cereal grains (Nature 159:513-514, 1947).

directly to the culture tubes without rinsing. In the first experiment, no effort was made to isolate seed from the floral tissues but the whole mass was placed upon the agar. In every case an *Alternaria* was found contaminating the culture but owing to lack of sugar fungal growth was slight. The roots of many of the plants were examined microscopically and in no case was any fungus-root infection found. The seedlings developed normally without fungal infection. In the second experiment, because of contaminations in the preceding, sound seeds were selected and the experiment was repeated. No one h.i.c. was found most favourable for growth but availability of iron was limited in more acid reactions. No root infection was found and the roots developed normally. "The conclusion is inevitable from this and the preceding experiment that the fungus is not essential for normal germination of the seed." KNUDSON supposed that RAYNER's uninfected seedlings had behaved "abnormally" either because of toxic action of a possible excess of iron in the culture solution, or through injury by mercuric bichloride in sterilisation.

RAYNER (1929) rebutted these arguments by stating first, that she never used more than a trace of iron in solution (3-4 drops of 0.1% solution per litre), and second that KNUDSON's sterilisation method (with calcium hypochlorite) was inadequate and that he had therefore not destroyed the fungus in the seed-coats.

FREISLEBEN (1933), finding the problem still unsettled, made culture experiments that demonstrated much better growth of *Calluna* seedlings with fungus than without, thus confirming RAYNER in respect to benefit of mycotrophy; but he also found it possible to grow sterile seedlings, indicating the symbiosis is not obligate and that mycelium does not penetrate seed. Indeed, he found much less general infection of the plant than indicated by RAYNER. Continuing his studies (1934), he found that endophytes of various *Vaccineae* could be exchanged, indicating that there was no obligate symbiosis in these plants. LUMIÈRE (1919) had called attention to the fact that STAHL had grown *Vaccinium* in sterile soil without difficulty. MOLLIARD (1937) studied *Calluna* still further and came to agreement with KNUDSON and CHRISTOPH, that presence of mycorrhizae is not necessary to development of heather.

Other Ericaceae:—In *Rhododendron,* infection by the endophyte is not an obligate condition of development of the higher plant, which in fact can form roots and establish itself in the total absence of micro-organisms (GORDON, 1937). Neither is there obligate symbiosis in *Azalea mollis:* MELIN (1921) experimented with this species and concluded that mycorrhizal fungi are not found in the aerial parts.

Freisleben's statements about *Vaccinium* have already been noted: he showed that in this genus there is no general shoot and seed infection, and accordingly no cyclic symbiosis can exist. In *V. macrocarpon,* according to Bain (1937), "Systemic infection of the type attributed to *Phoma radicis* by some investigators could not be found either in seedlings or in prepared slides from field grown material. The hypothesis of systemic infection by mycorrhizal fungi and its obligate relationship to root formation in the heath family was examined critically and it was shown that the hypothesis fails to conform to observed facts in some important respects, for example, in 100,000 cultures of cranberry fruits made by the U.S.D.A. in the last 30 years not even a single culture developed *Phoma radicis.*"

The casual nature of the mycorrhizal symbiosis is made evident once again by these studies. Apparently there is no obligate symbiosis in ericads in the sense that the ericad could not live without the fungus, neither that the two are habitually associated; but yet the fact remains that in the ericads' usual habitat, conditions exist which favour mycorrhizal association with an endophyte, and such exists. Perhaps in unusually favourable habitats there is a general systemic infection.

The Orchids:—Next may be considered the orchids, in reference to which there has been much argument as to their possible obligate mycotrophy. As to the possibility of its occurrence in roots and rhizomes of orchids, a denial was made a century ago by Reissek (1847): "The regularity and constance with which fungal formation occurs in orchids must be considered a characteristic and vital phenomenon, but just as phanerogams can be propagated without seed, so can orchids be produced without root-fungi."

In the orchid *Gastrodia,* Kusano (1911) concluded that the plant is dependent on its fungal endophyte because only in association did the orchid thrive and bloom well. But Curtis (1937) denies that there are any specific endophytes with orchids. He said: "There is an apparent correlation between ecological habitat and fungus type, rather than between orchid species and fungus." Bog orchids all had the same fungus indiscriminately but some orchids had different fungi dependent upon whether they grew in bog or prairie. Several strains of *Rhizoctonia* were isolated from the same orchid. Hence Curtis concluded that there is no specific mycorrhizal fungus, but any species found in the habitat may form mycorrhizae. This is the same principle of fortuitous mycorrhizal formation that seems generally to be true.

While speaking of orchids as a unit, it is evident that, like other sorts of biological phenomena, they consist of various discrete entities.

Burgeff (1932), who is so well qualified to speak on the subject, distinguished amongst the orchids, holosaprophytes (plants with normal roots but of a very limited number), hemisaprophytes (including only *Helleborine* amongst orchids), and root saprophytes (in which the root alone has taken over the saprophytic function of acquiring carbon-compounds). Root saprophytes, he said, seem less adapted to mycotrophy than rhizome saprophytes: *Gastrodia* is an extreme example of a root saprophyte. Saprophytism of the germinating plantlet, independence of the embryo of its nutrient tissue, the smallness and great number of the seeds, must go together with fungal infection and the antagonistic phenomena of phagocytosis. But determinate of results of infection is quality of the fungus together with the amount of its transmitted organic material. The fungi are of different sorts and lead by steps from *Rhizoctonia* to the cellulose-splitting Hymenomycetes and thence to the wood-fungi; and with increase in amount of nutrient translocated by the fungus there is development of saprophytism and increase in size of the saprophytic organ.

Germination of Orchid Seed:—Mention of the germinating plantlet leads to a consideration of obligate endophytism of orchid seed germination. Noël Bernard, the gifted French botantist who did so much for our science during his brief life, first made known the fungal symbiosis which exists with orchid seeds. It had long been a mystery why seeds germinated with such difficulty, although germination had been observed as early as 1804 by Salisbury, who described it from *Orchis Morio* and *Limodorum*. Bernard (1899), by a chance observation of germinating seeds of *Neottia*, a stalk of which had been accidentally buried in soil, saw that fungi (which he called "mycorrhizes") were associated, and jumped to the conclusion "that the mycorrhizae are indispensable to the plant at the time of its germination". This conclusion he then set himself to verify, with brilliant results. In 1900 he said: "The known difficulty in germinating orchid seed is due to the fact that presence of a fungus, normally present in orchid roots, is necessary for their development." In 1902 he stated: "In orchids the mycorrhizal fungus penetrates even into the germinating seed and apparently they are able to germinate only when infected." Next year he continued: "Thanks to the help of M. Magne, I am able to recount observations on germination of *Cattlyea* and *Laelia*. The seeds of these species and their hybrids are the most easily germinated of their series. . . . these experiments show that penetration of the fungus is *a necessary and sufficient* supplementary condition for their germination."

Necessity of Orchid Fungus:—Here was a thesis around which a battle was waged. BERNARD continued to develop his idea (1903): An orchid seed germinated aseptically, he said, will swell to a large diameter but will then still be stationary after 100 hours of culture, but transported to a pure culture of the hyphomycete will germinate at once. "This case is, I think, the first certain example of an organism which is not normally able to get past an embyronic state without penetration of a parasite, just as an egg, *e.g.,* cannot pursue its development without fertilisation. Using a term which has been applied to lichens, we may say that we have made *the synthesis of a plantlet of the orchid.*" These plantlets are not comparable to those of the majority of plants, which are derived from an egg, but are complex forms of the value of mycocecidia (*i.e.,* mycodomatia). BERNARD (1904) proceeded to isolate the orchid fungi, finding a number of which some induced germination and others not. In 1905 he recorded experiments showing orchids exceptionally difficult to germinate depend on endophytes different from those already isolated from *Cattlyea* and *Cypripedium*: thus there is a differential power amongst the fungi. He said (1909*a*): *Rhizoctonia repens* affects the majority of orchids but *R. languinosa* and *R. mucoroides* infect them with comparative rarity. (The orchid fungi are generally basidiomycetes: *cf.* DERX, 1937; SPRAU, 1937).

Degeneration of Orchid Fungus:—Then came a statement that added a fresh discussion: The activity of each species of fungus is not fixed but dwindles rapidly when the *Rhizoctoneae* live apart from the orchids: it does not require more than 2-3 years to cause their activity to become unappreciable. BURGEFF (1909), recounting his own experiments, stated, however, that while BERNARD found that orchid fungi, which are essential to germination of the seed, soon lose their virulence if cultivated for some time apart from the orchid, he himself found that the fungus of *Habenaria psychodes* and others, in spite of over 2 years culture on starch-agar exhibited the same infection-power which the fungus had earlier possessed. He felt that any idea of a decadence in infective power of the fungus should be abandoned. (It may be noted here that WOLFF, 1923, found that the age of orchid seed determines its power of germination, vitality dwindling rapidly after maturity and at the end of 38 months there was no germination.) BURGEFF isolated a considerable number of orchid fungi which he at first termed *Orcheomyces* but later called *Mycelium radicis.* That it is the orchid root fungi that cause germination of orchid seed was further demonstrated by SPRAU (1937), who isolated a *Rhizoctonia* from *Orchis masculus* that stimu-

lated seed germination of the same species. The same sort of relationship was supposed by WIBIRAL, 1910, to hold good with respect to the Gentians.

Hydrolysis of Starch:—In germination of an orchid seed (BURGEFF, 1910), at first some oil is transformed into starch; then the fungus grows in by way of dead suspensor cells, changes starch to sugar and thus increases osmotic energy and greatly accelerates growth. The fungus is now digested by enzymes produced by the orchid cell and a clot is left. With most orchid seeds, said BURGEFF, there is never germination without a fungus.

Sugars and Asymbiotic Germination:—In stating that the fungus changes starch to sugar another major thesis was enunciated. BERNARD had initiated asymbiotic orchid seed germination by causing the seed to germinate asymbiotically with salep, a product of dried orchid tubers rich in bassorin, a polysaccharide (*cf.* BALLION, 1924). BEAU (1920) had further experimented with seeds of *Spiranthes* and *Orchis,* causing them to germinate by transfer of salep material from gelatine to seed via a fungus. BALLION & BALLION (1924) secured strictly asymbiotic germination of *Cattlyea* with a medium half mineral and half organic. KNUDSON (1922) extended these experiments by trying the effect of various sugars on germination of orchid seeds. He sterilised seeds in calcium hypochlorite and cultured them on agar slants; and found that seeds of *Cattlyea, Laelia* and related forms germinate when sugar is supplied, fructose appearing more favourable than glucose. In glucose, chlorosis resulted, the concentration of glucose appearing important. Later (1924) he germinated seeds of various hybrids of *Cymbidium, Odontoglossum, Phalenopsis* and *Ophyrs,* finding "almost 100%" germination in sugar solution without fungal aid. He said: "These experiments lend further support to the hypothesis that the germination of orchid seed is dependent on an outside source of organic matter." Failure to germinate without sugar suggests necessity of an internal factor to activate chlorophyll, as indicated by BRIGGS. After the process is initiated, then seedlings develop without aid either of fungus or of sugar. In still further experiments, KNUDSON (1925) isolated orchid fungus resembling *Rhizoctonia repens* from *Cattlyea, Cypripedium* and *Epipactis;* and grew it in culture solution with added "0.5% starch". This fungus induced germination in *Cattlyea* "but not 100%", but there was no germination without it. The h.i.c. was increased by the fungus due to organic acids excreted by it, best growth being between *p*H 4.7-5.2. The fungus digests starch and changes it to sugar, while some of the

sugar is changed to organic acid. With less starch, most of the seeds were killed by the fungus. With starch in the culture solution there is no germination unless fungus is supplied. With sugar and fungus, plants made much better growth, the beneficial effect being attributed to the fact that the fungus changes the h.i.c. to a more favourable concentration. LaGarde (1929) found maltose the most efficacious sugar, and the best h.i.c. between 4.8 and 5.2, no germination taking place above *p*H 6.0. Clement (1924*b*) found that for *Odontoglossum* the *p*H was best held at 6.5-6.8, which he said possibly influenced solution of phosphates. For *Goodyera,* Downie (1940) said that levulose and dextrose are better than sucrose. But the same author said that seeds of *Goodyera* on mineral nutrient solutions adjusted to range of 3.6-7.6 failed to germinate without the fungus, but with the fungus no other aid was necessary. And Porter (1942) found the results of symbiotic tests were generally far superior to those secured on the asymbiotic substratum.

Fungus Supplies Sugar:—These experiments of Knudson's confirm earlier discoveries and further elucidate the fungus-orchid relation. It seems evident that the crucial action in germination of an orchid seed is provision of that seed with a sugar solution, although recent work indicates that something more than a carbohydrate is necessary for germination and development of the orchid, namely a "growth-factor" which is supplied by the fungus. Furthermore, Curtis (1943) thinks that vernalization is necessary for germination of *Cypripedium* seed: he secured some (20%) germination in an adjusted environment with a covering of agar to prevent a downward diffusion of oxygen. But provision of sugar can come only through aid of a fungus, or by artificial supply by man: if there is a third alternative we do not know it. Apparently the orchid seed is incapable of supplying its need through any autolytic action since practically all orchid seeds fail to germinate unless stimulated by an external aid as so early indicated by Bernard. Knudson fails to meet this situation when he asserts that fungi are not responsible for germination of orchid seeds; and his only suggestion is that in nature other fungi than the "orchid fungi" may provide the seed with sugar. But what other fungi?

Downie (1943) suggested that the symbiotic fungus of *Goodyera repens,* which cannot survive in compact humus, may live in other ways; for he found it on bifoliar spurs of *Pinus sylvestris* before the spurs were shed. He thought that his discoveries "contradict Knudson's hypothesis that orchid seed germination under natural conditions is effected by the action of non-symbiotic saprophytic fungi on the organic substratum".

Impotence of Unaided Small Seeds:—CEILLIER (1912) presented a suggestive paper on the germination of small seeds. In certain cases, such as in *Juncaceae,* the seeds are small and little differentiated but as they possess chlorophyll they are able to begin photosynthesis immediately on sowing. Small seeds with much reduced embryo also occur in parasitic forms such as *Cuscuta, Orobanche,* etc.; no fungus is present in these genera but apparently germination is not successful unless contact is made with organs of the requisite host. It may be, said CEILLIER, that the stimulus necessary in these cases is analagous to that requisite to bring about root-formation in those plants with "obligate mycorrhizae". Here is a suggestion of interest: *Cuscuta* can germinate unaided but only as it develops parasitic connections can it be nourished. By an apparently casual meeting with a host is its vigorous life assured. So it is apparently with the orchids; they have no specific fungi (as stated in some detail by CURTIS, 1939; also by DERX, 1937); but without any fungi at all they appear as helpless as *Cuscuta* without an host. This is more or less the conclusion of DOWNIE (1940) who used KNUDSON's methods with *Goodyera repens* and stated that "The experiments . . . tend to support the original contention of BERNARD that endophytic fungi play a large, if not the whole, part in germination of orchid seeds in the field."

Germination of fungus-spores, seeds and pollen-grains has been shown to depend on external supplies of activators, dependence on a particular activator being due to a failure to synthesize this or a similar substance (BROWN, Nature 157:65-69, 1946).*

Carbon Supply to Embryo:—Another angle of the problem was presented by POLLACCI & TREDICI (1936): It is not the fungus *per se* that causes germination but something produced by the fungus. Using seeds of *Cymbidium, Cattlyea* and *Phalenopsis,* he found that germination was accomplished by filtrate from *Rhizoctonia;* also from mycelium isolated from *Phalenopsis;* and perfect plants were grown to flowering age. What these substances are that produce germination remain to be discovered; but BEAU (1920) suggests that they are carbonaceous. He grew the orchid fungus on a gelatine to which salep was added and placed seeds of *Spiranthes* and *Orchis* on the glass but not in contact with the gelatine. When hyphae of the fungus grew up and into the seeds, germination was accomplished and growth continued until the hyphae were destroyed that connected the seed

*SCHAFFSTEIN "insists that seeds of the genera *Vanda* and *Phalenopsis,* for germination, must be supplied with the vitamin, vandophytin, which they lack" (Jahrb.wiss.Bot. 68:720-752, 1938).

with the gelatine; then growth stopped. This experiment indicated that sugar within the seed was not sufficient to support growth but that the embryo was dependent on an external carbonaceous supply. By further experiment it was found that the orchid fungus could grow "perfectly" on cotton moistened with non-carbonaceous mineral solution, the cellulose being dissolved. These facts fit into a carbon-theory of mycotrophy, and throw further light on the use of sugars in germination of orchid seeds.

Obligatism in Lower Plants:—The so-called lower plants are yet to be considered. In the hepatics, obligate symbiosis was posited by Chaudhuri (1925) for the Indian liverwort, *Marchantia nepalensis*. This species was found to contain an endophyte characteristically, but sterile thalli were easily raised, developing normally for a time but soon drying up without forming spores. Infested plants both vegetated and reproduced; from these facts it was concluded that the hepatic cannot develop to maturity without a fungus. Goebel's *Organography*, 3d Ed., remarks laconically: "This requires further proof". Nicolas (1924), working with *Lunularia cruciata* found likewise that fruiting occurred only in infected thalli, the sterile thalli being destitute of mycelium. This observation, together with Golenkin's, suggests the possibility, said Nicolas, that presence of a symbiotic fungus is necessary for fructification.

Chalaud (1932) considered the fungal endophyte necessary to tuberisation in the Indian hepatic, *Sewardiella*. Each year the successive gametophytes are infested but the endophyte is checked in the tissues by activity of meristematic cells that are incited to form an immune tuber. These facts, thought Chalaud, are in conformity with Bernard's discoveries about orchids; and he considered *Sewardiella* as the most nearly perfect adaptation of gametophyte to fungus amongst the liverworts, presenting a chart in which *Lunularia* infection is listed as accidental, *Marchantia* and *Pellia* as habitual, and in *Fossombronia* leading to a tardy tuber-formation and little change in vegetative character.

As to fern prothalli, Nakai (1933) supposed that uninfected thalli of *Cheiropleuria* would be sterile or male, implying a need for infection to form archegonia.

Infection of Lycopods is very general, but we do not know that the symbiosis has ever been claimed as obligate.

Summary:—From a review of what has been thought and discovered about obligate mycotrophy it is evident that there are two view-points in regard to the question, a theoretical and a practical view-

point. To illustrate them one may cite the following: A frog is killed and the heart bathed in an isotonic salt solution. One may now say the frog is not dead because its heart is still beating. In regard to Obligate Mycotrophy, there is an *academic* view of obligatism by which we have to admit that no plant is so united to its fungal partner that the plant cannot be forced to live in some other way; and there is a *natural* view of obligatism by which we perceive that the vast majority of plants in nature are obligatorily mycotrophic through their physiological requirements in a limited environment.

Added evidence for this view is afforded by the work of Dominik and Jagodzinski (Diary Trees & Forest Res. Inst. Kornik. 1 :48-73, 1946). Working with fruit trees, they find that certain spp. are confined to a mycorrhizal nutrition. Since the soils of Kornik garden contain less than a minimum required for ordinary plant nutrition, plants growing in it are necessarily dependent on fungi for N supply.

LECTURE XI

THEORIES OF MYCOTROPHY

Contrasted Concepts:—There are two contrasted concepts of the endophytic relation, first, that the fungus is a parasite on its host, and second, that the two symbionts live in some sort of a mutualism. Of 118 papers in which the writers expressed a definite opinion about the endophytic relationship, fourteen voted for parasitism. To some extent these opinions were based on personal bias rather than on experiment, and it remains evident that most investigators who have worked experimentally with endophytic structures conclude that the relationship of fungus to host is beneficial to at least the higher plant. But the views of the others deserve consideration, especially since in a number of cases the divergence in opinion is after all a divergence in viewpoint, and in the last analysis both sides are found in essential agreement.

Parasitism:—Those who concluded from a morphological study that the relationship is a parasitism include: CAVARA (1893), CHODAT & LENDNER (1896), GIBELLI (1883), GOEBEL (1888), MCDOUGALL (1914 *et seq.*), MASUI (1926), PRAT (1934), and ROBERT HARTIG (1888), who was decidedly opposed to FRANK's ideas of mycotrophism. He based his opinion chiefly on the alleged observation that trees in nature have young roots that are entirely immune to the fungus, and that mycorrhizae are more or less exceptional. "I have shown above", said HARTIG (p. 118) "That mycorrhizae are not always present, that not a trace of a mycorrhiza was to be found on 10-year old oak, beech, hornbeam, hazel, in the experimental garden of the Forest Institute, which were carefully excavated, that a relatively large number of roots of native trees which were carefully studied were found to be fungus free, and that there is not a single fact which would lead to the belief that cupulifers, conifers, ericads, etc., have so remarkably different a mode of nutrition from that of other trees. I see in mycorrhizal fungi nothing more than parasites which live on the tree but do not kill it, just as there are countless parasites which live on the leaves without injuring them." Much of the prejudice against mycotrophy which has existed to this day comes from this positive statement of HARTIG. Such is the power of dogmatism.

A number of investigators have concluded that the endophytic relation is a parasitism after experimental study. Thus, F. Fuchs (1911), who originated synthetic experiment in mycorrhizal study, came to the conclusion after studying synthetic mycorrhizae of conifers, that there is no symbiosis here of the sort in which the host plant is benefited. Where fungal hyphae penetrated into cells they were deformed and killed while the infested cells turned brown and were thrown off by the root. This holds good for endotrophic roots as well. The wide distribution of mycorrhizae indicates that it is an endured parasitism (ertragbares Parasitismus) in which the host plant suffers no injury because it is able to render the fungus harmless. With our present knowledge we can see that Fuchs was partly right and partly wrong.

H. Gordon (1937) was led to the view that the endophyte of *Rhododendron* spp. is a relatively feeble parasite and of no specific importance to the higher plant by the observation that in culture the higher plant can form roots and establish itself in the total absence of any micro-organisms. Needless to point out, Gordon worked only under the highly artificial conditions of culture and did not deal with actual plants in nature. Christoph (1921), like Gordon, concluded in the case of other ericads, that the fungus is simply a harmless parasite that does not injure the plant, because he was able to raise sterile plants in culture, and in nature he says that *Erica, Calluna,* the *Pyrolas* and *Monotropae* are only facultatively mycotrophic.

Curbed Pathogens:—Similar opinions are expressed regarding orchids. Bernard himself was no mycotrophist, but regarded the endophytes as invaders that are checked by an humoral substance in a phagocytosis that immunizes the remaining tissues of the plant. Costantin (1926) confirms Bernard and regards the orchids as pathological, being hereditarily accommodated to their disease. It was perhaps with such precedents in mind that Burges (1936) wrote: "The presence of a fungus in a mycorrhizal association is to be regarded as an example of controlled parasitic attack and has no mutualistic significance. The fungi are weak pathogens whose activity is curbed by the reactions of the host-cells. . . . One seems justified in concluding that the mycorrhizal fungi, both ectophytic and endophytic, are potential parasites controlled by reactions of the host-cells." Curtis (1939) likewise votes for parasitism, influenced by a cultural study of possible specificity amongst orchid fungi. From the fact that orchid seed can be germinated asymbiotically and that the orchid-fungus relation is non-specific, Curtis concluded "that the symbiotic relationship is one of parasite and host, with the orchid

deriving no benefit from the fungus in its roots." But MacDougal & Dufrenoy (1944) say that "The non-necessity of the fungus for germination of terrestrial orchids has been wrongly taken as a proof of parasitism by J. F. Curtis."

A similar comment could be made of Renner's (1935) assertion that because seeds of *Salix* and *Acer* germinate without fungal aid, also because soil-grown plants grew equally well without fungi while weaker plants in water-culture were killed by the fungi, that the symbiosis is a tolerated parasitism.

Magrou (1921) took the position that mycorrhizal symbiosis is on the border of disease. In perennial plants, as the potato, the fungus is in part phagocytized while in annual plants (as *Orobus*) it was completely destroyed. Limitation of the fungus was thought due to toxic constituents of the cell-sap, while tubers appear as symptoms of disease. In 1928, Magrou inclined to the view that the fungi are indifferent parasites which, if not harmful in all cases, are without any use.

Schools of Mycotrophism:—Those who reject the parasitic view of the mycorrhizal symbiosis and believe in mycotrophism are divided amongst themselves into several divergent schools of thought. They all agree that the mycorrhizal fungi are useful to the higher plant, but they disagree as to what the mycorrhizal fungi afford to the higher symbiont.

Mycorrhiza Replaces Root-Hairs:—One of the earliest views was that the mycorrhizal fungi, through the hyphae that connect mycorrhiza with soil, take the place of root-hairs and provide the higher plant with nutrient. Pfeffer (1877) said that in rhizome and roots of saprophytic orchids there frequently appears a fungus the mycelium of which, at least in *Neottia,* is found in living cells and sends out strands to the outside which, like root-hairs, take up organic and inorganic soil portions and with them make growth. Perhaps Pfeffer borrowed the idea from Drude (1873) who had said: "This constant entrance of a parasite into definite layers of the subterranean organs requires closer consideration. We have never found it in the epidermis: the question arises whether the basis thereof is not to be sought in the circumstances connected with the nutrition, whether perhaps the mycelial threads in these cell layers might not yield a rich nutrient." Marcuse (1902), after a study of representative mycorrhizae of a number of plants, came to the conclusion that "in most endotrophic mycorrhizae the communication-hyphae have a physiological role comparable to root-hairs, as was first hypothesized

by PFEFFER." KAMIENSKI (1884), finding *Monotropa* roots felted with hyphae which prevented any direct contact of the plant with soil, concluded that the fungus is its nutrient provider. "There is no other way by which nutritive solutions may pass and provide the roots of *Monotropa,* except the way of the mycelium." Fluid intake was also posited by ISSATSCHENKO (1913): "Disappearance of starch from the nodule (of *Tribulus*) was observed which, according to NOËL BERNARD, increases the osmosis of the cell and with it the water intake; and in spite of PAVILLARD's recent criticism, the author considers the relation between mycorrhizae and intake of water as unquestionable."

This was the idea which FRANK originally adopted. As reported by KLEBS, the fungus, which is to be regarded as a parasite in its colonisation and entrance, causes the host-tree no more damage than that an organ is developed which supplants the regular roots of the tree in taking in water and inorganic salts from the soil. As water cultures indicate, the tree is not dependent for its existence upon the fungus although it is evident that with fungal aid the tree grows all the more thriftily. Yet the fungus seems in its development confined to the tree, since efforts were not successful to grow the fungus on culture media. FRANK regarded his discovery of mycotrophy in cupulifers as "ein neues Beispiel von Symbiose im Pflanzenreiche" and stated (1885*a*) that "der Pilz als der alleinige Zuführer alles für den Baum erforderlichen Wassers und Nährmaterials aus dem Boden erscheint." This statement is simply an amplification of PFEFFER's earlier statement about *Neottia.*

MIEHE (1918) adopted the same idea when he said that mycotrophy is a more or less developed modification of nutrient salt acquisition.

Mycophagy:—But as FRANK continued to study mycorrhizae, he saw digestion of the mycelium and was led to develop another idea which may be termed "mycophagy". According to this concept, the "fungus-eating plants" are able to draw their victim into the protoplasm, there to tend it and make it large, and finally to digest it, and thus the rich protein production of the fungus is made use of. One of the two symbionts seems to have the advantage of the other in that it appears as the raw material for the other. Stripped of its fattening-pen implications, this concept has persisted. It was adopted and modified by MAGNUS (1900) for *Neottia:* So far as purely anatomical structures indicate, the physical significance of the digestion-cells consists in an exclusive use for the higher plant by which the substance-rich fungus is killed, digested and excreted; the

significance of the host-cell is an exclusive use for the fungus which grows there purely as a parasite, injures the protoplasm, forms "closed" organs which apparently serve to overwinter the plant. It was adopted by KUSANO (1911) who said that the fungus, although it acts as a parasite at times, becomes a victim of the orchid so that the reciprocal exchange is not equal, for "*Gastrodia* is parasitic on the fungus". And CORTESI (1912) believed the relation between fungus and orchid is a case of helotism in which the fungus plays a subordinate role. The orchid supports and nourishes the endophyte so long as its presence is beneficial but it finally kills the fungus when flowering and seed-production time arrives.

PRAT (1934) came to the same conclusion about *Taxus canadensis*. Symbiosis, he said, is scarcely the term to be used but disease, for the invading fungus is limited by the host; and thus the tree becomes a parasite on its parasite! For *Empetrum,* HASSELBAUM (1931) concluded that there is no question of a mutual symbiosis, but rather an attraction and destruction of the endophyte; and FRASER (1931) regarded two species of *Lobelia* studied as parasitic upon their mycorrhizal fungus. For *Vinca,* DEMETER (1923) saw an advantage to the host plant, according to FRANK's concept, in mycophagy by which the host makes a certain gain in N; but he did not regard the symbiosis as ideal since the host-plant may be badly injured. Besides, under cultural conditions the fungus-free plants made decidedly better growth than the infected, indicating the mycorrhizal relation was more of a parasitism.

Romellian Hypothesis:—In contrast to mycophagy is ROMELL's (1939) hypothesis: that "the obligate mycorrhizal fungi associated to conifers are not saprophytes decomposing soil organic matter, and that they are energetically parasites on their host trees." MACDOUGAL & DUFRENOY (1944) take an opposite view-point, stating that "The absorption of inorganic phosphorus from the soil by the fungus and the stages of its metabolism terminating in the stele of the root, identifiable origination in hyphae and translocation of auxin, vitamins and amino compounds to the root tissues, together with the capacity of isolated segments of mycorrhizal roots to survive and grow, like a chlorophylless plant, establishes the non-parasitic character of the fungus." BJÖRKMAN (1944), furthermore, found that on so treating pine seedlings that they ceased to form carbohydrate in the root, the fungus did not become a parasite: it simply ceased to "attack" the root.

Nitrogen Theory:—It is FRANK's nitrogen theory that has been received with most attention, a theory that is briefly stated by LINDQUIST (1939): The nitrogen theory was first clearly formulated by FRANK (1894), [in a short discussion of the various possibilities of significance of mycorrhizae, in which he supposes the fungus makes available the N-compounds contained in forest humus and duff. More probably, it was said, the fungus aids intake of humic compounds of calcium], who considered that the beneficial influence of the mycorrhizal fungus consists mainly, for the higher symbiont in the provision of organic N-compounds for the latter. As basis of this conception, FRANK, etc., pointed out that the tissues of a mycorrhizal tree are nitrate free. As it is known that the fungus can readily take up NH_4 and organic N-compounds, he considered it self-evident that such compounds were taken up from nitrate-free or nitrate-poor forest soils and into the mycorrhiza-bearing tree. The N-nutrition hypotheses were extended later by numerous successors, as *e.g.* VON TUBEUF, MÖLLER, MÜLLER, WEISS, and especially MELIN, who in the years 1917-1927 conducted numerous investigations into the mycorrhizae of forest trees and their rôle as N-absorbing organs. . . . Without wholly renouncing the mineral nutrition hypothesis, he assigned to the mycorrhiza no great rôle in the intake of mineral nutrients, showing in this connection that the trees have ability to obtain rich nutrition from the mineral soil by their deeply penetrating roots.

A similar reciprocity was claimed by RAYNER (1927) for *Calluna,* and she said: "it seems probable that MELIN's conclusions respecting the beneficial effects resulting from the presence of the mycorrhiza in acid humus may be extended to the more specialized case of *Calluna.* Under such conditions, the mycorrhiza of this species, and doubtless of other ericoids, probably functions in a similar way to that of trees, conferring on the host plant the power of drawing upon the organic food reserves locked up in humus. . . . Like certain conifers and other trees, *Calluna* and its allies are not strictly autotrophic in respect to their nitrogen metabolism, and they are singularly well equipped for successful competition in the struggle to obtain the requisite nitrogenous food materials, whether in sandy soils, poor in organic constituents, or in acid humus soils deficient in nitrates. They have solved the problem of growth upon the poorest and most unpromising soils, but they have solved it at the price of their independence."

Chemical Studies of the Nitrogen Theory:—We come next to a chemical study concerning presence of NH_4 salts in plants by WEEVERS (1916), who found NH_4 salts present in all species investigated except mycotrophic and insectivorous sorts. He said that

mycotrophs apparently make use of organic N compounds through aid of their mycorrhizae, this action taking place best on acid soils, the occurrence of mycotrophs on alkaline soils therefore becoming impossible.

Weyland (1912) initiated micro-chemical studies of the occurrence of salts in tissues of mycotrophic plants, and concluded from his studies that, as a result of a limited transpiration stream which results in part from activity of the fungus, P and K would be developed in concentrated form in the plants. The plants could thus satisfy their Ca-requirement only by living on a calcareous soil from which they evidently take the Ca. The theory of winning nutrient salts through fungal aid, which the author (Weyland) had learned from his teacher, Stahl, broke down at this point. Nitrogen (Harnstoff) in orchid tubers was regarded as a metabolic product of the root fungi. N-assimilation is to be considered as an essential function of the root-fungus.

An extended micro-chemical study of mycorrhizae was made by Rexhausen (1920) who, seeking particularly to test Weyland's conclusions, found that mycorrhizae were as rich in P and Ca as were the fungus-free roots; also in protein content, although it seemed somewhat greater in infected plants. He said that mycorrhizae are to be seen as isosmotically acting individuals which provide the plants with all nutrient salts, apparently not merely P and Ca. He thought that the fungus takes N from the higher plant because of the ease of acting as a parasite in comparison with difficulty of securing nutrient salts from the humus. Only in soils rich in nutrient for the fungus can the higher plant throw off the fungus. But he was sure of the nutrient salt provision for he repeats: "In the other plants studied it may be stated positively that nutrient salts are brought into the root through the hyphae."

Finn (1942) found in culture studies of white pine that seedlings provided with mycorrhizae took in more N and K per seedling than those of uninoculated controls. Earlier, Mitchell, Finn & Rosendahl (1937) had reported that their observations "indicate that the benefits attributable to mycorrhizae, like their distribution in nature, vary inversely as the concentration of readily available mineral nutrients in the soil. Seedlings lacking mycorrhizae are unable to exist in very infertile substrates."

Importance of P:—In some cases at least, P seems of greater importance than N. Thus McComb (1943) states that with pines, especially *P. Banksiana,* good growth followed P fertilisation while little or no response was obtained with N. "Without mycorrhizae

pines acquired P with difficulty." "It is suggested that the stimulating effect of mycorrhizal fungi on conifer seedlings is due to heightened metabolism, associated in this instance with transfer of phosphorus and growth stimulators from fungus to seedling" (McComb & Griffith, Plant Physiol. 21:11-17, 1946). Young (1940) attributes the benefit to the fungus, for "With the presence of adequate P for fungus growth and the availability of a phosphatide supply, the fungus is enabled to carry out the breakdown of raw organic matter and transfer the products to the higher plant." Young presents a diagram to show the effect of phosphate applications on the nutritional cycle of the mycorrhizal fungus and the pine tree. Intake of P is important to the higher symbiont in formation of phosphoric complexes as seen in the next lecture.

Stahlian Hypothesis:—"The mineral hypothesis was first definitely formulated by Stahl (1900). He considered Frank's conception of a close relationship between mycotrophy and N-supply to be false and claimed that, in soils poor in mineral nutrients, trees are brought into competition with fungi and bacteria; and through mycorrhizal symbiosis the tree is benefitted. Stahl's hypothesis has not met with favour amongst modern investigators except with Hatch, who goes beyond Stahl in claiming the mycorrhizal relation to be chiefly a 'physical relationship', *i.e.*, the chief significance of the mycorrhiza is in its increase in absorbing surface."

Hatchian Hypothesis:—Hatch (1937), however, said his hypothesis was "tentative" and applied only to "ectotrophic" mycorrhizae, and stated: "The mycotrophic relationship in pine, and presumably in other plants possessing ectotrophic mycorrhizae, is a symbiotic mechanism which increases, chiefly by physical and therefore by relatively non-selective means, the absorption of soil nutrients. . . . The greater absorption capacity of mycorrhizal seedlings is brought about by, and is proportional to, increases in the effective absorbing surface areas of short-roots resulting from fungal invasion. . . . Trees are dependent on symbiotic association with mycorrhizal fungi for their soil nutrients and therefore for their existence in all but the most fertile agricultural soils."

Routien & Dawson (1943) sought to amplify the Hatchian hypothesis, and after experimenting concluded that mycorrhizae increase the salt absorbing capacity of the roots primarily by adding to the supply of exchangeable H-ion derived in part at least from carbonic acid. They found that development of mycorrhizae increased the average rate of aerobic CO_2 production of each short root from

nearly 2-4 times the normal amount depending upon the degree of such development.

The Hatchian hypothesis suffers from two grave disabilities: (*1*) it fails to take into consideration the rooting medium of the mycotrophic plant. The loci of mycorrhizae are most diverse and in many cases are disintegrating organic residues, and it is rather puzzling to understand how there can be a total intake of water and mineral salts from a medium consisting chiefly of organic compounds. (*2*) The Hatchian hypothesis (and all other hypotheses of mycotrophism for that matter) does not make provision for an intaking mechanism. Advocates of the root-hair hypothesis of plant nutrition have considered the root-hair in detail, and in so far as root-hair nutrition occurs the process is fairly understood; but advocates of mycotrophy blithely ignore the structure which transfers materials from the soil to the interior of the mycorrhiza, and leave one to assume that in some way the substances jump in. The mycorrhiza apparently says: "Abracadabra", and the deed is done!

Transpiration and Mycotrophy:—It must not be lost sight of that the essential concept in Stahl's hypothesis, especially in the eyes of his contemporaries, lay in the relation of mycotrophy to transpiration. To Stahl, mycotrophy was most necessary to those plants which have a limited transpiration stream and accordingly obtained less nutrient salt in a "normal" way. A large stream of water passing through the plant and being transpired from the appropriate organs would presumably leave large quantities of salts in the tissues. Recent work indicates that plants which transpire for long periods with little elongation of the roots take in water through lenticels, breaks around branch-roots and wounds (Addoms, Plant Physiol. 21:109-111, 1946). Plants which have a limited transpiration stream and hence more difficulty in securing nutrient salts are those which have "sugar leaves" rather than "starch leaves". The advantage of starch formation lies in the lessened trouble in assimilation and the greater ease of transpiration, while addition of sugar increases the difficulty of the latter. Thus, plants which secure nutrient salts with difficulty are those which do not excrete liquid water from the leaves; and of these Stahl listed a number. Stahl perhaps got his idea from F. Schwarz (1883), who said that conifers and cupressineae have the leaf reduced and cuticularized, and hence have a lesser transpiration stream: in these trees root-hairs are lacking and the water and salt requirements are met by parasites (Schmarotzern).

Burgeff (1909) adopted Stahl's views on transpiration in orchids, apparently without much thought; while he found feminine

supporters in Busich (1913) for asclepiads, and Kerkichu (1930) and Stajanow (1916) for orchids. Stahl's idea in regard to orchids was that the orchids can take in and transpire only a small amount of water but the symbiotic fungi have a greater osmotic power and the orchids owe to this property a greater inflow of water and the salts dissolved therein.

Objections to the Stahlian Hypothesis:—Opposition to Stahl's view developed. Tubeuf (1903) pointed out that Stahl's view could not apply to endotrophic mycorrhizae because, he said, there is no connection of the endotrophic mycorrhiza with the surrounding soil, meaning apparently that individual hyphae are insufficient to meet the needs of the plant. Stahl was at fault, too, in regard to liverworts; for Němec (1899) had asserted that *Marchantiaceae,* being starch producers, could not have endophytes, which were to be expected only with the sugar-producing *Jungermanniaceae;* and Stahl had seized upon this suggestion as fitting into his hypothesis. But actually, endophytes are commonly present in the Marchantiaceous liverworts. Then, as to orchids, Fuchs & Ziegenspeck (1925) pointed out that in orchid mycorrhizae the transformation of cortical cells into digestion-cells interferes with water-transport, hence lessened transpiration is not compensated by mycotrophy but is rather caused by it.

Opposition to Stahl's hypothesis developed also in regard to his ideas in relation to soil sterilization. Existence of a struggle for nutrient salts was posited amongst roots of all plants, and mycotrophic plants were supposed to gain an advantage over concurrent fungi through aid of mycotrophic fungi. The existence of such a struggle was thought by Stahl to be demonstrated by the fact that autotrophic plants do better in sterilized than in unsterilized soil. But Neger (1903) conducted experiments that led him to conclude that thriftier growth of plants in sterilized soil is to be attributed mainly to the increase in nutrient salts caused by sterilization and not (or only to a minor degree) to the misconceived battle against soil fungi.

Björkman's Hypothesis:—Coming still closer to an explanation of mycotrophy, Björkman found a connection between the products of photosynthesis in the host and mycorrhizal formation. A vigorously growing plant in an environment moderately deficient in N and P, or both, and exposed to optimum illumination forms abundant mycorrhizae because there is abundant reserve of assimilate in the root tissues for the mycorrhizal fungus to use. The author's only

acquaintance with this hypothesis is through ROMELL's (1944) review, from which the following is quoted: "the mycorrhizae seek soluble carbohydrates in the root, and they consume them for energy, but they find them only if there is a surplus of carbohydrate in the root. Whether or not there is any surplus depends on the head-start photosynthesis (carbohydrate formation) has attained in the plant before the formation of albuminous substances. This advantage will be small or nil if there is an abundance of all plant foods including nitrogen and phosphorus, so that there will not need to be a shortage of materials when albuminous substances are built up. Then there will be little for the fungi to seek after in the root, and mycorrhizae will be formed sparingly or not at all. On the other hand, should there be a deficiency of nitrogen or phosphorus foods, there can easily arise a surplus of carbohydrates so that the formation of albuminous substances falls behind photosynthesis. With a moderate lack of nitrogen or phosphorus or both, mycorrhizae can form abundantly, but if there is a serious lack of nitrogen or phosphorus, sooner or later photosynthesis will become weak also (phosphorus especially strongly influences carbohydrate formation in plants) so that the surplus of assimilated carbohydrate becomes less for that reason, and mycorrhizae are formed more rarely. Similar reactions occur if light becomes weak. *Mycorrhizal formation is, expressed briefly, a result of and a sign that there is a certain surplus of energy-giving nourishment in the host plant.*"

Carbonaceous Theories:—Besides the theories of mycotrophy which posit the intake of inorganic salts, there are not lacking theories that connect it with carbon. The idea that roots take in carbon has been familiar from the days of LIEBIG and his "humus theory" which posited a normal intake of carbonic acid by rootlets of seedling plants. The same idea appears in a paper by BRÉAL (1894) who, in an experimental study concerned chiefly with cereals, concluded that these plants are able in effect to take up organic carbon materials through their roots. BEAUVERIE (1902) studied the liverwort, *Conocephalus,* and found that endophytic infection is most pronounced where humus is most abundant, and where humus is lacking there is no mycelium and thallus is small sized. By experiment it was indicated that the plant secures part of its C from the humus. For the orchid *Gastrodia,* MCLUCKIE (1923) came to the same conclusion: "*Gastrodia* is an elaborate example of symbiosis in which an Angiosperm is associated with a fungus and a bacterium; and is directly or indirectly dependent upon its endophytes for its carbonaceous and nitrogenous foods." Other examples are given by YOUNG (1940).

Hydrocarbon Hypothesis:—That nutrient exchange in mycorrhizae is carbonaceous rather than nitrogenous was advocated by McLennan (1926). "A critical discussion of [some of] the earlier work on mycorrhizae, more particularly that dealing with the physical relations between the two forms, discloses the fact that the most generally accepted ideas of this relation are those connecting it with nitrogen fixation without any real evidence that such is the case. . . . The demonstration of many infecting strands [in *Lolium* mycorrhizae], together with the appearance of fat, firstly in the conducting and travelling hyphae of the root, with its subsequent removal to the sporangiole, and then to the host-cell, accompanied by collapse and shrivelling of the fungal mechanism, have led to the conclusion that a metabolic exchange takes place *from the fungus to the higher plant, with the result that the latter obtains a supply of fat or oil.*" Knudson's results were thought to favour this hypothesis and "The idea that the exchange is carbonaceous rather than nitrogenous is also compatible with Bernard's suggestion" of the relation between tuberisation in plants and the presence of endotrophic mycorrhizae.

As a postscript we may add: "Although these homogenous globules do not stain black with osmic acid after bichromate, they have, nevertheless, been proved to be fat globules."

Carbohydrate Hypothesis:—Another carbonaceous hypothesis was proposed by Young (1940) as a "mycorrhizal theory regarding the cause of fused needle disease" in pine. "According to this hypothesis, normal mycorrhizas supply the tree with an essential part of their carbohydrate supply, and it is to the inefficient functioning of the mycorrhizas in this respect that the fused needle condition is due. The supplying of additional phosphorus to soils low in this element results in a more abundant phosphatide excretion from the pine roots, thus stimulating normal mycorrhiza formation and bringing about a satisfactory balance of conditions for correct mycotrophic activity. The amount of vegetable detritus present is important in this respect, as it is from this source that the carbohydrate supplied to the higher plant by the mycorrhizal fungus is obtained. The addition of phosphates to the soils in question stimulates the growth of natural vegetation, and thus aids the development of the necessary supply of vegetable detritus."

This explanation was not accepted by Neilson-Jones (1941), who ascribes development of needle fusion to a sudden shortage of water in the plant as the leaves start to expand, due to a failure to produce mycorrhizae at the critical juncture. But the hypothesis of carbon intake through mycorrhizae remains to be experimentally

evaluated. Young (1941, p. 91) makes it clear that it is not merely C that the plant receives from the soil through its endophyte but "Inorganic salts and perhaps nitrogen compounds are probably also supplied to the plant."

Growth-Promoting Substances:—In more recent years attention has been turned to growth-promoting substances, and Lindquist (1939) presented a Growth-substance Hypothesis, based on his studies which indicated an excretion of a substance from mycorrhizal mycelia that stimulated growth of spruce in pure culture. "The mycelia . . . influenced so markedly the nutrient liquid into which they were drawn during these experiments that a decided increase in growth of needles, as well as of stem and root, could be marked." Lindquist considered it established that "the nutrient materials derived from fungi are of essential, indeed of vital, significance." Burgeff (1934) also found the higher plant stimulated by the fungus, in this case specialized orchids of the *Vanda* group, which always develop slowly in absence of their natural symbionts. In promoting growth, the dead fungus was found to be just as effective as living hyphae, because of presence of a "growth-factor" resembling "Bios II" although its chemical nature was otherwise unknown. In significant experiments, Noggle & Wynd (1943) tried the effect of various growth-promoting substances, and found "good germination and excellent development of the seedlings when nicotinic acid (P - P factor) was supplied in the nutrient medium."

But Romell (1939) rejected Lindquist's hypothesis that "the chief function of mycorrhiza formation would be the exchange of growth-promoting substances between the symbionts", and "returned to Frank's original ideas". Yet it is established that growth-promoting substances do influence mycorrhizal fungi. Melin (1939) found increased growth with aneurin in synthetic (glucose-containing) medium; yeast filtrate gives even better results while biotin and inosit failed to improve growth except in combination with aneurin. Aneurin (vitamin B_1) also gave better growth when added to cultures of endophyte of *Arum* (Magrou, 1939). Later, Melin (1942) found that aneurin is replaceable by its pyrimidin and thiazol components, and equimolar quantities in synthetic cultures of certain mycorrhizal fungi. The development of *M. r. atrovirens,* which is aneurin-autotrophic, is retarded by addition to the medium of aneurin or its components, especially pyrimidin alone or mixed with thiazol. Experimenting with orchids, Meyer (1944) concluded that thiamin (vitamin B_1) substantially aided growth of seedlings.

Working with aqueous extracts of fall-litter, MELIN found that "all types of litter contained substances soluble in water which favourably influenced mycelial growth of thiamin-heterotrophic fungi (mycorrhizal Hymenomycetes and Gasteromycetes, as well as litter-decomposing Hymenomycetes) in a nutrient medium containing sugar, salts and thiamin. The litter extracts likewise exercised a favourable influence on the development of thiamin-autotrophic soil fungi." These results apply only to tree-litter examined and not to litter from the grass, *Glyceria*. The litter extracts appear to contain growth substance like ROBBINS' "Z" factor (Symbol. Bot. Upsaliensis 8(3):1-116, 1946).

Limitation of Mycotrophic Hypotheses:—It must be realized in any study of mycotrophic hypotheses that the protagonists of each hypothesis have been specialists in some one field of mycorrhizal study, and their hypothesis naturally reflects experiences with their own material. It may be that each investigator is correct, as undoubtedly he is so far as his understanding of his own data is concerned, but no one person has yet had a broad enough grasp of all phases of mycotrophy to develop an explanation which will fit all cases.

Again, it must ever be remembered that a mycorrhiza is not a static thing: ENDRIGKEIT (1937) has neatly expressed the case: "Since the parasitic acquisition of nutrients by the fungus is of a very restricted order in both mycorrhizal groups, the higher plant is evidently the chief gainer by the association until the activity of the roots begins to decrease with age, the balance inclines in favour of the fungus. Comparative membrane and permeability observations on plants in a colonized and uncolonized condition revealed a progressive loss of independent assimilatory capacity of the roots with increasing fungal activity."

The Intaking Mechanism:—The point at which current theories of mycotrophy break down is with respect to the mechanism for intake of liquid materials from the habitat. It is after all of little consequence whether the plant is to receive N or P or any other ion if there is no means of transport for these substances. Of course the plant does receive them, but not much attention is paid to the method or the apparatus by which the substances enter the mycorrhiza or mycotrophic organ. The presumption is that they enter through communication-hyphae, but so far as we are aware there is not a single research devoted to the structure and functioning of these hyphae. They seem to have been forgotten by the protagonists of

the various mycotrophic theories; yet they are utterly essential to any theory of mycotrophic nutrition. To formulate a theory of nutrient intake and ignore the intaking apparatus is like baking a cake—with the baking-powder omitted.

Ectotrophic Intake:—It is evident that there are two very distinct sorts of mycotrophy, as has been indicated from early days. In the first sort, the ectotrophic (if there is indeed a true ectotrophic mycorrhiza), the surface of the mycorrhiza is covered with a fungal mantle from which extend out numerous hyphae that assume various shapes, sizes and forms. This mechanism would seem to be suited to an intake of liquid materials,—water and dissolved salts,—just as Frank originally observed. Yet even among these mycorrhizae there is a great difference between various sorts as briefly but well shown by Woodroof (1933) for pecan. Here the surface of the mycorrhiza is in some forms covered with short setae that seem to have the qualities of root-hairs and may function as such.

Root-Hairs Versus Setae:—In any report of root-hairs on an otherwise mycorrhizal root, a due scepticism must be maintained until there is positive proof that the structure is a root-hair and not a fungal hypha. Short-roots are frequently to be observed with what appears to be a firm epidermis from which extend trichomes in the form of root-hairs, but on closer inspection it is to be seen that the "epidermis" is composed of closely appressed hyphae from which setae extend. Thus, MacDougal & Dufrenoy (1944) remark that the mycelium of the endophyte of *Corallorhiza* sends "branches outwardly through the epidermal cells of underground coralloid branches in simulation of the arrangement of root-hairs." In cases of doubt the material must be embedded and sectioned; and lacking such demonstration we remain unconvinced in reading any report of the presence of root-hairs on mycorrhizal roots.

Endotrophic Intake:—For the endotrophic mycorrhiza there is a very different structure. There is no mantle of mycelium, no mycoderm, no setae; and the only hyphae that connect the mycorrhiza to the soil surrounding are the so-called communication-hyphae. If water and salts are to enter the mycorrhiza they must come through these hyphae or through the uncuticularized portions of the exposed root. But could substances come through these hyphae? Recall that the endotrophic mycorrhiza is the prevalent sort, the one by far the most common, especially if we consider the functioning of the ectendotrophic mycorrhiza as in part included here. Recall also that the

hypha is in effect a capillary tube of relatively great length. Now it is true that water passes readily through a capillary tube, but only if it is empty. The fungal hypha is not empty but filled with a more or less viscous colloid protoplasm. Furthermore, in the hymenomycetous hypha there are septae at intervals, in the soil portion of the mycelium, at least, although cross-walls are rare inside the mycorrhiza. (It is to be noted that MacDougal & Dufrénoy [1944], states: "Pads of material at the pores of cross-walls of hyphae . . . were observed. Possible similarity of composition and functioning with sieve plates of higher plants is noted.") Here there are two obstacles to the passage of liquids through the "communication-hyphae",—colloid and cross-walls; and unless different physical principles can be adduced than those which ordinarily obtain, we can only conclude that the "communication-hypha" is not an efficient apparatus for transfer of water and mineral salts, if it is used in that way at all.

"Individual hyphae, as the demonstrations of Frank indicate, can not suffice for extensive transportation paths" (von Tubeuf, 1903). "The endotrophic mycorrhizae have only a slight independent capacity for absorption as compared with the autotrophs." (Endrigkeit, 1932).

Hyphae as Nutrient Conveyors:—Contrast the "communication-hypha" with the root-hair. The latter is constructed for intake of liquids: It is a thin-walled cell-extension, relatively short and of considerable diameter as compared with its axial length. It has a minimum of protoplasm and contains a large sap vacuole that is so constituted as to aid osmotic action. The communication-hypha is the opposite to all this: Its wall is not of cellulose; it is tremendously long in comparison with its diameter; it is densely filled with protoplasm with a minimum of vacuole, and it may even be boxed off at intervals by septae.

Obviously, the communication-hyphae function otherwise, namely in bringing in of elaborated organic substance to the mycorrhiza where it is dealt with in an ordinary biological way.

Teleology in Mycotrophism:—In spite of scientists' sensitive denials of teleology, the current theories of mycotrophy are decidedly teleological. The mycorrhizal apparatus is represented in some sort as an automat wherein the mycorrhiza drops a starch-grain in the slot and takes out a dish of nitrogen compounds or of phosphorus salts. It is the author's opinion that ordinary processes of biology are sufficient to explain the mycotrophic reactions without attributing needs of salt absorption, etc., which the higher plant seeks to satisfy; and this opinion is elaborated in the next lecture.

LECTURE XII

MYCOTROPHIC PHAGOCYTOSIS

Significance of the Term:—In 1905 BERNARD had arrived at the conclusion that orchid "symbiosis is in some sort a serious and prolonged disease, intermediate between a fatal malady and complete immunity." This idea was elaborated in 1909: BERNARD sought to show, as VUILLEMIN said, the common characters between infectious diseases of animals and those of plants, and the narrow bonds which unite the states of symbiosis and disease. He transported to botany the medical terms of vaccination, immunity, phagocytosis, etc. The word "phagocytosis" connotes three distinct actions, *viz.* (*1*) Attraction of foreign bodies, (*2*) their capture or active penetration in the element into which they are drawn, (*3*) their intracellular digestion and assimilation. But for BERNARD the word "phagocytosis" implied nothing more than intracellular digestion; and he dissociated the first two acts from the third. He regarded the *Rhizoctoniae* and their orchids as two antagonists (1909*b*), developing their means of attack and defense; while symbiosis (*i.e.,* mutualism) represents an immunity attained by phagocytosis. The plant makes use of all its means of defense in order to preserve its essential tissues.

After this vitalistic statement, the mechanics of the reputed defensive action are related. The formation of mycelial coils in orchids, of GALLAUD's arbuscles in mycorrhizae of *Allium,* of JANSE's sporangioles in roots of plants in Java, are all considered phenomena of agglutination due to a humoral property of phagocytotic origin. But BERNARD did not overlook the fact that clotting of hyphae took place in cultures of *Rhizoctoniae* where there could be no question of lytic action induced by an orchid tissue. BERNARD's untimely death put a stop to these significant studies.

It should be noted that there is a distinction between phagocytosis, which is probably a proteolysis in large part, and toxicity. MACDOUGAL & DUFRENOY (1944) neatly contrast this difference: "The action of the 'humoral' secretion . . . is to be distinguished from toxicity by the fact that in the first case the secretion simply blocks some of the processes of cell metabolism but does not destroy the mechanism; in toxic action (as in pseudomycorrhizae) a theoretical secretion causes irreversible and fatal changes in the cytochemical set-

up of living cells of the organism affected. In parasitism, in addition to possible anatomical and cytological damage, a fatal extraction of nutritive material may ensue."

Older Descriptions of Phagocytosis:—New names do not make new processes, and that of phagocytosis had long been known, although not under that name. Reissek (1847) observed ellipsoid bodies in *Neottia* that later turned yellow,—in other words, clot-formation; and in *Orchis Morio* he noted the process was especially active in autumn and early winter. He described and figured peloton formation with remarkable clearness. Schacht (1854) saw that the fungus uses starch in orchid rhizome and root-cells; but Prillieux (1856) differed with Schacht: The 2-3 outermost cortical layers of cells in *Neottia* were filled, he says, with a yellowish-brown matter which Schacht had considered as an index to death of cell. Prillieux found it in a great number of orchid genera, in cells which retain their nuclei; but he noted that the matter seemed to diminish in roots which have vegetated a long while, from which he inferred that the brown matter served for nutrition of the plant. The material, he said, is probably nitrogenous. The infected orchid cell nuclei are of great size and often have 2 nucleoli. The cells having brown matter regularly contain filaments wound without order about the central mass in each cell. Reinke (1873), noting the matter or "slime" in orchid cells, ingeniously supposed that it is a "Schwellkörper" or apparatus for maintaining sap concentration.

Phagocytosis in Peloton Mycorrhizae:—The process of phagocytosis in the peloton mycorrhizae, found chiefly in orchids, is accordingly as follows: Hyphae penetrate in part through root-hairs or in part directly through cell-walls, and enter the cortex, finding entrance through passage cells where an exodermis is present. Hyphae pass freely through cell walls, a marked constriction sometimes being evident in the hypha at the point of passage of cell-wall (Dangeard, 1898) which grows up about the hypha (Burgeff, 1932) to form a wall-tubule or "Röhrentüpfel." This organ Burgeff considered one which, by differences of suction force, passes water or solutions of low sap concentration through the hypha. Passage of hyphae through cortical cell-walls is sometimes prevented by thickening of the walls to 6 or 7 μ, by undergoing an excessive cuticularization (Burges, 1939).

At a certain distance from the central cylinder, depending on the sort of orchid, the hypha ceases to grow forward and begins to coil. Burgeff (1909) observed coiling on aerial hyphae when a drop of

water was excreted: a hypha being drawn into the drop would be prevented by surface tension from growing out of it again, and would thus continue its growth by coiling within the water drop. Perhaps something of the sort occurs in orchid cells, those cortical cells nearer to the central cylinder having an higher concentration of ions in the cytolymph and accordingly a greater surface tension in the protoplast. Regardless of explanation, the hypha coils in cells of the cortex, the nucleus of the cell remaining intact but becoming enlarged, hypertrophied and even dividing amitotically. According to most authors, hyphae never penetrate raphide cells but Busich (1913) says that the fungus is not warded off by calcium oxalate but on the contrary forms it.

Later the hyphal coils degenerate and release their content into the host-cell. "As with many other orchids, the process of ingestion of the hyphal coils is preceded by a turning point at which the h.i.c.

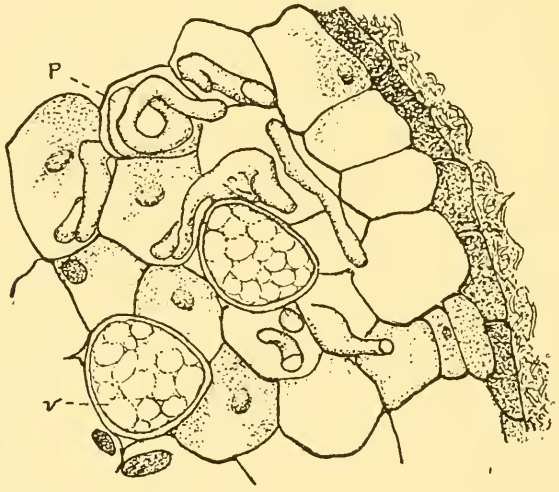


Fig. 12.—Portion of a cross-section of the ectendotrophic mycorrhiza of *Cornus florida,* showing hyphal coils or pelotons (p) and vesicles (v).

of the hyphal clumps reached a maximum of *p*H 6.2." (Hamada, 1939). The actual breaking down of the hypha seems due to the action of a proteolytic enzyme (Burges, 1939) and results in the formation of a more or less homogeneous yellowish mass in the centre of the host-cell. This is the "yellow body" or "matière brunâtre" or "gelbliche Stoffe" of earlier investigators. The yellow

clots are highly refractive, of irregular form, and are very resistant to acids and alkalis. In concentrated H_2SO_4 they dissolve after a few days, while in KOH they swell somewhat, and on treatment yellow drops appear on the periphery which with osmic acid turn dark brown, indicating presence of fat or oil (Wahrlich, 1886).

The material brought into the orchid cells has been termed proteinaceous, and the hyphae were called "protein hyphae" (Eiweiss-hyphen), but according to A. Fuchs (1924) they are actually glycogen hyphae. The breaking down of these hyphae, according to Burges (1939) is due to a loss of vitality. The addition of small quantities of extracts from tubers, stems, leaves and roots of orchids to young cultures of the endophytic fungus resulted in all cases in an inhibition of fungal growth and complete decomposition of hyphae within 3-4 days, root extracts being much less toxic than those prepared from tubers or stems. Sap of host-cells, withdrawn by means of a micropipette and added to blocks of agar smear-cultures produced visible changes within 24 hours, and at the end of four days some empty hyphae could be seen.

Phagocytosis in Arbuscular-Vesicular Mycorrhizae:—Mycotrophy in this sort of mycorrhiza was described by Boulet (1910) from cultivated fruit trees. He said the habit of the endophyte is very constant: Mycelium traverses the piliferous layer, penetrates into cortical cells, ramifies, but seldom penetrates further than ¾ width of the cortex. The endophyte apparently lives upon the starch reserve of the cells which harbour it, for the starch reserve disappears from these cells. In the most internally placed layers part of the hyphae continue development in the cells while another part insinuate themselves into the intercellular spaces, filling the cavities. The mycelium frequently contains reserve. In the region of the endodermis, which is never penetrated, the hyphal wall is partially dissolved and inclusions extravasated. On certain filaments vesicules are abundant and some are intercellular (size 100 μ x 54 μ) while others are intracellular (57 μ x 36 μ). The intercellular hyphae, by a more or less regular dichotomy, form dense coralloid branches called "arbuscles" by Gallaud. The branches often terminate in sporangioles which at times are so numerous as to fill the cell cavities. They disorganize rapidly to a granular, somewhat floccose, mass and finally into scattered granules; or, even more frequently a solid mass is formed and degeneration seems checked.

Janse, who invented the name of "sporangiole", speaks of them as present in a number of the plants he describes; *e.g.*, in *Dysoxylum*, a member of the *Meliaceae*, he said the fungus penetrates to the cor-

tex, showing swellings before it passes the cell wall and later branching to form terminal sporangioles which at first are filled with spherules, and finally to a gummy mass. Nuclei of cells which contain them are much larger than those of other cells nearby. Again, in *Begonia robusta,* the fungus produces sporangioles which at maturity contain very fine granules which finally diffuse through the cell. In *Helicia* (of the *Proteaceae*) large cortical cells are infected by hyphae that form sporangioles and later free their content to form a gummy mass.

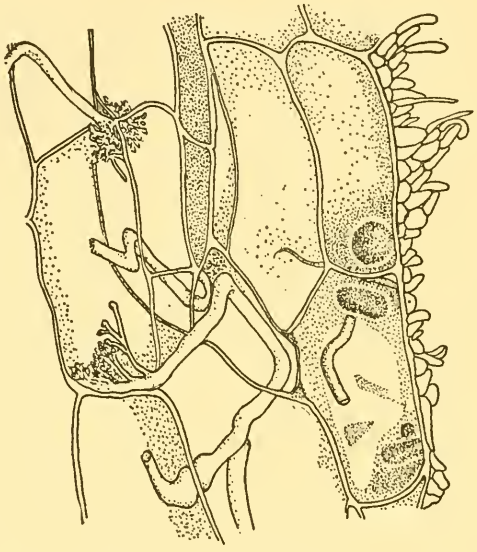


Fig. 13.—Portion of a section through mycorrhiza of *Abies balsamea* indicating some of the mantle or mycoclena to the right, and within, fungal hyphae and arbuscles.

In *Vitis* (Petri, 1907) the intracellular mycelium shows the following development: (*1*) A net of fine hyphae is formed about the starch grains, which are soon dissolved. (*2*) Nuclear elements in the ends of these fine hyphae undergo a differentiation which recalls the synkarion-phase of the basidia during karyogamy. (*3*) A great quantity of proteinaceous material accumulates in the ends of these hyphae. (*4*) By chemical transformation, these hyphal branches and their content are gradually transformed into prosporidia.

In asclepiads, Busich (1913) found a special sort of vesicle termed a "glomerule"; and she also found formation of vesicles outside the root. In *Vinca* (Demeter, 1923), penetration of the epider-

mis is direct and the fungus passes through "Kurzzellen" or passage cells into the cortex where, in consequence of presence of fungus, the starch dwindles. Inter- or intra-cellular hyphae become swollen or wurst-shaped, either terminal or intercalary. In age, in addition to a number of nuclei, they have great fat vacuoles with protoplasm between, like cross-walls. When intracellular, vesicles are always terminal, and the small glomerules described by BUSICH were never found by DEMETER. The content of the vesicle may be resorbed into the hypha as there is seldom a cross-wall evident; and the function of the vesicle is apparently to serve as a temporary storage organ. "Germination" of the vesicles has been reported, said DEMETER, by BERNARD and BUSICH only; and the very infrequence bespeaks a slight significance for the germination. But more important in the lives of both symbionts are the arbuscles, and both the simple and the compound sorts described by GALLAUD are found in the *Apocynaceae* and *Asclepiadaceae*. Simple arbuscles are always terminal, formed at the end of hyphae which have penetrated cells. In the end branches in young stages of arbuscles there are little granules arranged in nebulae, and staining deeply with haematoxylin. From their origin these nebulae appear to be protein precipitates. As a result of action of free H-ion in the cell-sap, the tips of the arbuscles burst and empty their content into the host-cell. It is possible, said DEMETER, to form these "plasmoptyse" of the endophyte in pure culture, the action occurring in vitro at an optimum acidity of 0.025 N HCl, and on the basis of this observation, the name of "Plasmoptysen mycorrhizae" was chosen. "Sporangioles" are merely the last structureless residue of the arbuscles which have been made harmless, a residue which is finally resorbed.

Just as the hyphae can be broken down in HCl and the content extruded as in the "plasmoptyse", so in pure culture DEMETER found that by use of different concentrations of sugars he could produce peculiar stuntings of growth which recalled arbuscle formation.

Phagocytosis in Ericaceous Mycorrhizae:—KAMIENSKI (1884), in his pioneer work on *Monotropa,* saw that the roots of this plant are much branched and interlaced, and fragile; the epidermis, being covered by a fungal mycelium of septate hyphae that form a pseudoparenchymatous mantle two or three times as thick as the epidermis. The fungus lives on the surface and never penetrates living cells except sometimes in older portions where those cells are filled with a brown "tannin" content. In older parts of *Monotropa* roots the epidermis disorganizes at the same time as the mycelium develops. It is evident that the epidermal cells play an important

rôle, for all the interior cells are cut off from the exterior by a fungal mantle: "Consequently, there is no other way by which the nutritive solutions may pass and provide the roots of *Monotropa* except the way of the mycelium. This is composed of vegetative filaments of which those that neighbor the epidermis are applied so closely to these cells that diffusion seems not only possible but absolutely exists. *Monotropa",* said KAMIENSKI, "is thus able to nourish itself by the mediation of this fungus."

But MACDOUGAL (1900) said that in the *Monotropas,* vesicles, sporangioids and sporangioles fill the cells, and "probably serve as organs of interchange". FRANCKE (1934) said that infection is

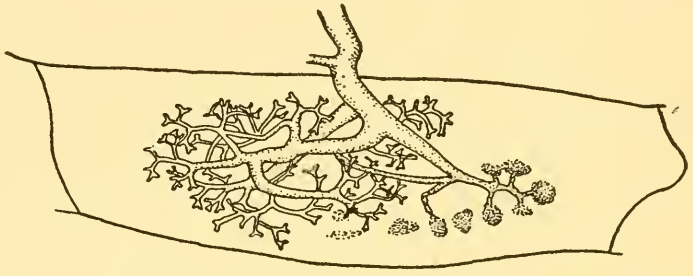


FIG. 14.—A cell from mycorrhiza of *Allium sphaerocephalus* showing an arbuscle which is breaking down to form sporangioles (*Redrawn from* GALLAUD, Rev. gén. Bot., 1905).

limited to the epidermis, one hypha only penetrating a cell, going to the nucleus where it forms a plasmoptyse, followed by phytophagy in which there is no evidence of excretion. No great changes were observed in the host nucleus.

In *Empetrum* (HASSELBAUM, 1931) digestion also begins next the nucleus and proceeds outward. During digestion the cell nuclei become amoeboid, its nucleoli are split up and yielded to the plasm as producers of ferment. A distinction between host- and digestion-cells is observable, the cells being of different morphological origin: in the host-cells the fungus forms thick coils of hyphae which are not digested while in the digestion-cells there is a sporangiole mycorrhiza "destined to digestion".

In *Calluna,* RAYNER (1927) described intracellular digestion of mycelium. "Throughout the growing season the mycorrhiza cells exhibit active intracellular digestion of mycelium with disappearance of the resulting—and presumably soluble—products. The nearer to the apical meristem, the more rapidly is digestion initiated. Its onset is marked by the usual signs of cell activity—increase in size and

chromatin content of the nuclei often accompanied by deformation, 'clumping' of the mycelium about the nucleus, disappearance of the sharp outlines of individual hyphae, and the gradual conversion of the hyphal constituents from the region of the nucleus outwards, to a structureless mass possessing strong stainability. The last stages in the process are marked by shrinkage of the nuclei and disappearance of the stainable contents. This intracellular digestion of mycelium is a continuous process observable throughout the vegetative season from early spring to late autumn. The proportion of cells in the active mycelial condition or undergoing digestion at any given moment varies with the time of year, the age of the root, and possibly also with the season and other external factors."

Phagocytosis in Ectotrophic Mycorrhizae:—LAING (1923) makes the statement that there is no evidence of digestion in ecto-

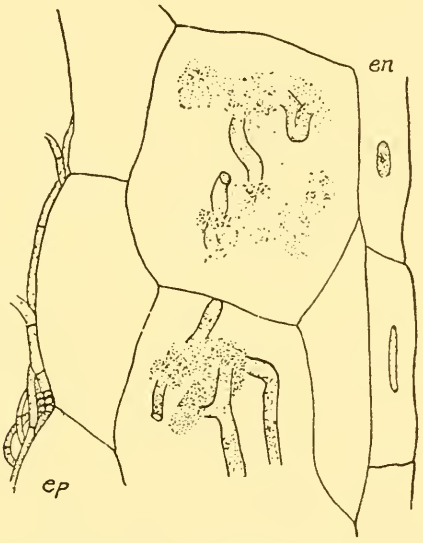


FIG. 15.—Portion of a longitudinal section through a mycorrhiza of *Pteridium aquilinum*, showing a plasmoptyse stage in breaking down of hyphae (*Redrawn from* LOHMAN, Univ. Iowa Studies in Nat. Hist., v. 9, pl. II, fig. 10).

trophic mycorrhizae of conifers. But the whole question of ectotrophism remains in doubt for if it is true, as MELIN (1923) remarked, that in older researches the delicate intracellular hyphae may

have been overlooked, then there is no such thing as a true ectotrophic mycorrhiza with the mycelium closely surrounding the rootlet but not penetrating its cells. Were such a mantled root to exist as indicated, then mycotrophy in such a case would inevitably consist in a provision of the higher plant with materials taken directly from the soil (since the higher plant is otherwise isolated from the soil by felted hyphae) while the fungus would gain nothing except a congenial site for mantling its hyphae. But if the "ectotrophic" mycorrhiza actually has hyphae extending into the host-plant's cells, then its mycotrophism is the same as for other sorts of mycorrhizae, namely a mycotrophic phagocytosis. In the absence of definite information our judgment must remain suspended; yet we can make one incidental observation, that free-hand sections are useless for mycorrhizal study and researches based on this method are necessarily invalidated.

If we distinguish a mycorrhiza as "ectotrophic" when it possesses an Hartig net and ignore the question of infection or non-infection, then the cases described by MELIN as ectendotrophic may be utilized for this category. In *Larix* (MELIN, 1922*b*), three phases of mycorrhizal formation are distinguished: (*1*) The fungus penetrates intracellularly into the roots and forms individual hyphae or knots; (*2*) then the intracellular hyphae are digested and the mycelium penetrates intercellularly, while (*3*) finally the fungus lives almost exclusively externally and the mycorrhiza becomes mainly "ectotrophic". In *Pinus sylvestris* and *Picea Abies* (MELIN 1922*a*) the hyphae grow principally in the interior of the cortical cells where they form a pseudoparenchyma of the same appearance as in the fungal mantle of the completely developed mycorrhiza. Later the Hartig net and the fungal mantle are formed.

In *Betula* and *Populus,* MELIN (1923) described the mycorrhiza as consisting of (*1*) an hyphal mantle; (*2*) a "palisade" layer in which there is an Hartig net and intracellular hyphae of two sorts: (*a*) Haustorial hyphae which are very thin (1 μ) and grow in a tortuous course: they are seldom septate, are plasm-poor and sometimes fragment while at other times they form grape-like bodies. (*b*) Protein (Eiweiss) hyphae may attain 10 μ thickness. They extend longitudinally in the palisade cells and grow into neighbouring digestion cells or penetrate several palisade cells. They are at first very rich in plasm and protein and contain several (up to 8) large (3 μ) nuclei which have apparently 12 chromosomes. They seldom branch. (*3*) Digestion layer, which is bounded by an endodermis provided with tannin and starch wherein is no infection.

Melin concluded: "The anatomical structure shows that the higher symbiont suffers no injury from the fungal hyphae. Quite the contrary, some of the hyphae are later digested, whereby the higher symbiont obtains some nutrient while the fungus, through its haustorial hyphae, derives some nutrient-material from the higher symbiont. Finally, a nutrient-interchange takes place between the Hartig-net and the palisade layer which long keeps both tissues alive".

These descriptions inform us of the ectotrophic (or ectendotrophic, if we choose) mycorrhizae in Sweden. From the other polar

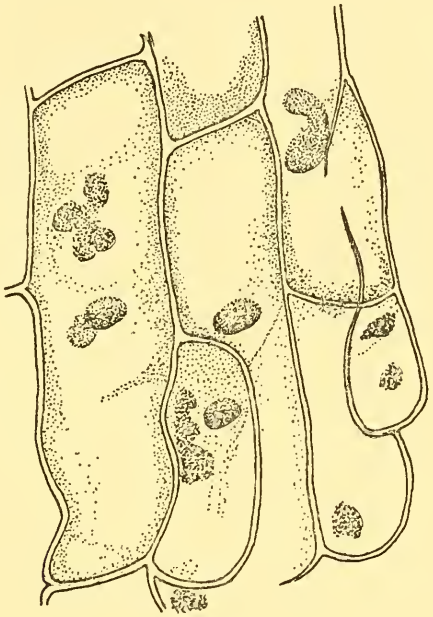


Fig. 16.—Some cells from mycorrhizal cortex of *Fraxinus americana,* in which the fungal reserve, which overwintered, is largely broken down and partaking of a plasma stain. Note enlarged nuclei.

extreme, from the Cape of South Africa, comes an exactly similar report. Smith & Pope (1934) state with reference to mycorrhizae of *Eucalyptus*: All the main internal features (the layered mantle, the palisade-like epidermal cells with "Hartig-net" mycelium) are paralleled in Melin's descriptions of other tree mycorrhizae. The fungus is usually present inside cells of epidermal layer and the outermost cortical layer but rarely occurs in any deeper layer. Intra-

cellular digestion of hyphae is exhibited with a clarity unusual in tree mycorrhizae.

Endrigkeit (1937) says that at no time is there intracellular digestion in *Pinus*. In the monograph on mycotrophy in *Pinus* (Hatch, 1937), we learn nothing of the method of intake of nutrient, the mechanism of intake, or of possible phytophagy.

Limitation of Endophyte:—Confinement of the endophyte to a certain region of the mycorrhiza is a common observation. It was the basis of the early distinction between ecto- and endotrophic mycorhizae, the former having the endophyte supposedly confined to the epidermis of the host. In those mycorrhizae in which hyphae penetrate internally, Frank (1885) observed that they never go beyond the innermost cortex of cupulifers that are invaded. In fruit-trees the hyphae penetrate three-fourths of the distance through the cortex (Boulet, 1910). In *Olea* the "prosporidi" are localized in an internal zone of large cells of cortical parenchyma (Petri, 1908). McDougall (1914), in studying forest trees of Illinois, found that the central cylinder is never invaded; while *Taxus* in France is said (Prat, 1926) to keep the fungus out of stelar tissues by a layer of "tannin" in the endodermis. In *Eucalyptus* the fungus is found in epidermis and outer cortex but rarely deeper (Smith & Pope, 1934). Of particular interest, remarked Noell (1910), are cases like *Cunninghamia* in which hyphae penetrate only a few certain cell-layers without any reason being apparent why they should not invade all the cortical cells. Even the fossil tree, *Amyelon,* shows the central cylinder never penetrated (Halket, 1930).

Such phenomena were freely recorded by Janse (1897), whose work is characterized by so much admirable detail: In *Ophioderma,* sporangioles are found in third layer of cortex only while in *Lecanorchis* it is the second layer that is invaded, and in *Dendrobium* all layers except the last are penetrated. In *Burmannia* the layer next the endodermis is exempt while in *Aronychia* the hyphae never invade the innermost cortical cells, which are filled with "tannin". In *Elaeocarpus,* invasion is to the mediocortex only while in *Michelia* invasion is confined to certain points in the cortex, and resin canals are never penetrated. So, too, in *Dysoxylon* the secretory canals are never invaded.

Limitation in Orchids and other Herbs:—Besides the orchids named by Janse, the following may be cited: In *Centrosis* it is the mid-cortex to which the endophyte penetrates and the inner cortex and the central cylinder are always free from infestation (Arcu-

LARIUS, 1928). In *Pogonia* the fungus seems never to penetrate deeper than the inner cortical cells (CARLSON, 1938). In *Neottia* the 3-4 outer layers of cortex are infested (MAGNUS, 1900) while the fungus never penetrates the central cylinder which, said MAGNUS, "seems a remarkable localization". PITTMAN (1929) found that the fungus never penetrated *Rhizanthella* tubers. AMES, who saw (1922) that the vascular tract of *Goodyera* is never invaded, remarked (1921) that certain areas of the orchid root (*sic*) seem able to repel advance of the fungus; and "it is as if there were some fungicidal capacity in the cells of the root structure that restricts the fungus to a limited area."

Some other herbs may be mentioned: Thus, O'BRIEN & NAUGHTON (1928) found the fungus in localized patches of inner cortex in *Fragaria;* and TREUB (1885) said that in *Saccharum* the central cylinder is never invaded. For the ferns the same condition obtains: In the sporophyte of *Botrychium,* at a definite distance from the epidermis, the fungus branches copiously and forms sporangioles while the vascular tissue is free. In the gametophyte the outer cells are at first invaded but become fungus-free, which is the condition of the apex and reproductive organs at all times. In *Ophioglossum* prothalli the inner cells are fungus-infested while the outer are free (BRUCHMANN, 1904; LANG, 1902). In *Lycopodium* the fungus is present in epidermis of prothallus only (HOLLOWAY, 1920), or at most 1-2 outer cell layers (GOEBEL, 1887).

Supposed limitation of endophytic invasion by what are called tannin deposits does not occur, for the endophyte can freely invade such cells. Incidentally it may be remarked that WOODROOF (1933) found tannin formed in cold weather and present in both mycorrhizal and non-mycorrhizal roots.*

Limitation in Hepatics:—Every report on the hepatics indicates a definite localization of the endophyte. Thus, in *Conocephalus* the fungus is limited to a zone of central tissue (BEAUVERIE, 1902), while GOLENKIN (1902) reports that in a number of liverworts the hyphae are confined to a compact ventral tissue. In the New Zealand liverwort *Monoclea* the fungus is found in a sharply defined zone and does not occur in the growing point (CAVERS, 1903). In *Marchantia* the fungus is limited to a zone beneath the air cavities (CHAUDHURI, 1925), while in *Lunularia* the endophyte is present in a band of tissue (EMBERGER, 1924; NICOLAS, 1924) along the midrib (RIDLER, 1923). RIDLER

*MACDOUGAL & DUFRÉNOY state that decompensated respiration results in polymerization of the quinoids into gummy masses, the presence of which forms a barrier to the extension of hyphae (Plant Physiol. 21:1-10, 1946).

(1922) said that the fungus occurs in a definite zone along the ventral midrib of *Pellia* and that the hepatic seems to exercise control over the fungus. According to Magrou (1925) the fungus degenerates about the archegonia and sporogonia, which seem to exert an inhibitory influence upon growth. Auret (1930) found further that the endophyte does not penetrate gemma-cups and archegonia of *Lunularia*. Moreover, in *Sewardiella* (Chalaud, 1932) the fungus is checked by active meristematic cells and the bulb is immune.

Limitation in Root Apices:—From all published accounts the mycorrhizal apex is free from infection. To give some examples: No hyphae were found in the vegetative point of *Hippophaë* (Arcularius, 1928); the root tip of *Vitis* is never invaded (Petri, 1907), nor the apical meristem of *Taxus* (Prat, 1934); while in pecan only occasionally does the fungus enter cells of the growing tip (Woodroof, 1933). In *Neottia* the fungus is always found a short distance back of the growing point (Drude, 1873), while in *Philesia* the fungus penetrates to within 10-12 zone cells behind the apex (Macfarlane, 1897). Young roots of *Paris* are fungus-free 1.5 cm. from the root-apex (Schlicht, 1889). In *Monotropa* the fungus diminishes toward the apex (Kamienski, 1884); it does not enter the meristematic zone of *Dipodium* (McLuckie, 1922); and the root-tip of *Thismia* is fungus-free (Pfeiffer, 1914). The fungus is seldom closer than 3-4 mm. of the root-tip of *Corallorhiza* (Thomas, 1893); in *Angiopteris* and other ferns the endophyte is absent from the root-tip (West, 1917).

Limitation in Long Roots:—It is well-known that the long roots of extension are fungus-free. Thus Gibelli (1883) said that in *Castanea* the long, rapidly growing roots are free from infection, while in *Cacao* the long roots are specifically stated by Pyke (1935) to be fungus-free, and they are rarely infected in *Taxus* (Prat, 1926).

Limitation in Green Tissues:—Magrou (1925) noticed that when hyphae invade cells of *Pellia* containing chlorophyll, the latter is destroyed; and Ridler (1922) also observed that chloroplasts disappear in *Pellia* on fungal invasion. Conversely, where chloroplasts exist there are no fungi: Thus, Bolleter (1905) found that green plants of *Conocephalus* showed no infection while neither starch nor chlorophyll bodies occurred in infested cells. Again, in *Lunularia*, chlorophyll tissue is never invaded (Emberger, 1924); and indeed, Golenkin (1902) had said that in liverworts infested cells never contain starch or chlorophyll. Moreover, where *Orchis incarnata* roots

were exposed to light, chlorophyll developed on the upper (lighted) portion and here there was no infection, but in the lower (shaded) portion chlorophyll was absent and the endophyte was present (Burges, 1939). This observation had been anticipated by Janse (1897), who noted that *Lecanorchis* cells were fungus-free when they contained chlorophyll. Mollison (1943) suggested a loss of fungal vitality after a length of time, to explain failure of fungus to penetrate where chlorphyll is developed.

Summary of Limitation:—The sum of all these observations is as follows: The invading endophyte is kept out of mycotrophic plant tissues (*1*) at a definite distance from stelar tissues of vascular plants; (*2*) from the growing apex of the root, never occurring in a meristematic tissue; (*3*) from all chlorophyll tissues, which of course contain active plastid bodies; and (*4*) from reproductive bodies such as gemmae-cups, or archegonia of liverworts. Or to sum up these categories into a single one, the endophyte is kept from all places where active physico-chemical processes occur. They are kept out by what has been aptly called a brutal phagocytosis.

The Starch Relation:—One more link in a chain of evidence must be presented, namely, the fungus-starch relation. Briefly, fungus and starch stand in inverse relationship, for where the fungus is present no starch exists, for the fungus utilizes the starch as it progresses. Many examples may be cited: Boulet (1910) found that starch disappears from fruit tree mycorrhizae when fungus is present; Ruggieri (1937), that starch vanishes from sporangiole cells of *Amygdalus;* Endrigkeit (1937) noted similar disappearance of starch from *Rhamnus;* Figdor (1897), from *Cotylanthera;* Issatschenko (1913), from *Tribulus;* and Jennings (1898), from *Corallorhiza.* Starch disappears from *Dipodium* mycorrhizae soon after penetration of hyphae (McLuckie, 1922); the fungus uses starch in *Centrosis* (Arcularius, 1928); penetration of hyphae in *Orchis* is followed by dissolution of the starch (Fuchs, 1924); on the entrance of the fungus into *Pogonia* the starch begins to disappear (Carlson, 1938). Burgeff (1909) had said that the orchid fungi dissolve out starch as they go, a statement anticipated by Schacht in 1854.

On the other hand, Kusano (1911) stated that in *Gastrodia* starch disappears from all mycorrhizal cells of the cortex but reappears in the inner (third region) after cessation of metabolic activity. In the innermost cells of *Ophioglossum* prothallus the fungus is absent and cells are full of starch. In *Botrychium* the apex and reproductive

organs (being fungus-free) are full of starch, which occurs nowhere else. In *Lycopodium,* infected cells contain oil rather than starch (Bruchmann, 1906), while in *Pellia* (Ridler, 1922) the fungus uses starch "which is replaced by oil after entrance of fungus". Again, in higher plants it is found that "Whereas in non-infested roots the starch is deposited indiscriminately, in those colonized by mycorrhizae it preponderates in the cells free from mycelium" (Endrigkeit, 1937). Added evidence that carbohydrates are used by the fungus is provided by Björkman (1944) whose experiments show that pine on being "strangled" by a wire placed 5 cm. above the ground level formed almost no mycorrhizae while the amount of soluble carbohydrate in the roots dwindled. Björkmann believed that mycorrhizal form is largely conditioned by an excess of soluble carbohydrate in the roots. The fungus can use only a readily soluble carbohydrate like glucose, as shown by Melin & Norrkrans (1942). Magrou has found that formation of potato tubers is conditioned by the osmotic pressure of sugar within the cell. In nature, it is the mycorrhizal fungus which ordinarily changes starch of the plant cell into sugar. "Ce processus de dislocation des parties colloïdales du protoplasme à été désigné par Errera sous le nom d'anatomose" (Ann. d. Sci. nat. Bot., XI, 4:97-102, 1943).

Rexhausen (1920) has summed up the matter by saying that the fungus takes carbohydrates from the plant in the form of sugar. Thus sugar is obviously obtained by use of the plant's reserve starch. Or, as MacDougal & Dufrenoy (1944) state: "Hydrolyzation products of polyuronides, of starch, and of other diffusible compounds may be absorbed by the fungus."

At the same time Young's (1940) objection must be taken into account: "The concept of the higher plant obtaining carbohydrate from its fungus symbiont is in direct contradiction to the unsupported but generally assumed theory that the mycorrhizal fungi obtain carbohydrate from the tree roots as their share of the symbiotic relationship. The hymenomycetous fungi which form the mycorrhizas are, however, quite capable of obtaining their own carbohydrate supply from the breakdown of organic matter. This is evidenced by their vigorous growth on raw organic substrata and is supported by the experimentally proved fact that one of the major functions of the fungi associated with orchid roots is to supply carbohydrate to the higher plant." The solution of this problem would seem to lie in this, that the fungus "dissolves" starch as it invades the tissues of the higher plant, and releases later to the higher plant whatever it has brought from the soil on phagocytosis. The action would seem to be in both cases mechanical.

Conclusion:—The explanation of these phoenomena remains for the future. Certainly there is an underlying cause. Meanwhile, certain facts may be pointed out.

(1) It is already established that there is a difference between the included content of stele and cortex. MacDougal & Dufrénoy (1943) state: "The pericycle and endodermis layers in the root mark the boundary between two contrasted types of tissues; those in the stele rich in phosphorus linkages which may be described as energy-rich, and able to counterbalance the oxidase activity, and those in the cortex, relatively poor in such linkages, and rich in catechol and in catechol oxidase. Fungi . . . never transgress beyond the endodermis into the stele." Further, these authors state: "Oxidase activity seems to be higher in the cortex of the pine root (whether previous to mycorrhizal infestation or after) than it is in the stele. Such a difference should play a role in controlling selective permeability: anions, with their negative charge, should be carried from the site of higher activity, to that of the lower. The tissues of the stele, from their meristematic stage, maintain a low oxidase level, by retaining a high level of phosphoric complexes, acting as dehydrogenases. This condition enables them to trap such anions as (H_2PO_4) or (HPO_4)".

(2) Another fact is, that the hypha which penetrates into the cortex develops branches at a certain point to form an arbuscle. Such proliferation is ordinarily the result of introducing an hypha into an hypertonic solution of ions. We may note that Burges (1939) had already noticed that during early stages of infection hyphae are capable of further growth but that as histological changes become apparent the fungus gradually loses its vitality. "The intracellular arbuscles cannot be interpreted as assimilatory organs, since they are digested as they form and show no indication of hyphal development from their terminal branches, but rather as proliferations induced by the growth-promoting stimuli of the cell-sap" (Endrigkeit, 1937). Vuillemin, in reviewing Gallaud's work, said that arbuscles are less a characteristic production of the endophyte and more a result of the reaction of the host-cells to invasion by a foreign body. Magrou (1939) saw incipient arbuscle formation with endophyte of *Arum* on addition of aneurin. Demeter (1923) had found with endophyte of *Vinca* that peculiar stuntings of growth, called forth by different concentrations of sugar and special sorts of sugar, recall arbuscle formation.

(3) The hyphae break down. By dissociation of complexes in the cell-sap, free H-ions are left in solution. These ions, acting on the fungal arbuscle or the undifferentiated hypha, cause it to break down and extrude its plasm into the host-cell. Demeter (1923) had shown the breaking down of hyphae in vitro at an optimum acidity of 0.025N HCl. This concept is in agreement with Magrou (1921), who said that the fungus is limited to certain parts of the plant through toxic constituents of the cell-sap. It is also indicated by Hatch's (1937) statement that susceptibility to infection by mycorrhizal fungi is apparently controlled indirectly by the internal concentration of nutrient elements in short-roots. These "toxic substances" are apparently ions normally present and not special humoral bodies. Routien & Dawson (1944) suggest an increased H-ion output in the mycorrhiza, arising from carbonic acid, but leave unsettled the question of its origin.

(4) The fungal material is digested. The presence of proteolytic enzymes enables the host's digestion-cell to utilize the extravasated plasm of the fungus. Hitherto the hypha was utilizing the host's substance; but now the host gets back not only what it had previously lost but all that the fungus brought in from the soil. In this sense there is a total intake of mineral salts, organic substances and water by the mycorrhiza, but all combined as protoplasm of the fungus.

(5) The digestion-area is strictly localized. Since the ionizable substances which pass from the stele to the cortex are subject to definite physical laws, the rate of diffusion is specific for a given sort of plant, being conditioned by the nature of the substances through which diffusion must take place. For this reason, phagocytosis must necessarily be limited to a certain region of the cortex. "I think," said Emberger (1924), "that localization of infection is conditioned by differences of osmotic pressure."

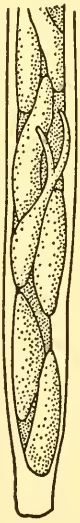
The apical meristem and other growing points are richly supplied with ionizable substance by the flow of liquid materials into such regions. Through these rich supplies, actively growing tissues can readily repel the endophyte by breaking it down at a distance from the meristem to which the ionizable substances extend. Chloroplasts in green tissues and perhaps leucoplasts in tubers probably exert a similar influence.

(6) The mechanism of phogocytosis is apparently ionic. A plant is not static, and the more active its growth the more ionizable material it will have at its disposal, and the more certainly will the fungus be destroyed in its tissues. Hence it may be understood what Reed

& Frémont (1935) discovered when they applied stable manure or cover crops to plots of *Citrus* and found that the trees seemed to develop resistance to the fungus, a resistance which untreated trees seemed to lack. In the treated trees they found that the cytoplasm of the host-cell enveloped arbuscles of the fungus with apparent active proteolysis. It is evident that with better conditions of growth the *Citrus* trees had more H-ion at their disposal for breaking down of the fungus.

In this connection likewise may be cited the writer's (1944) studies of chestnut, sprouts of American chestnut having little resistance to blight whereas seedlings have decided resistance. Moreover, seedlings under better conditions of growth in a natural woodland are more resistant than seedlings in the open. Resistance to the fungus is once again, in these observed cases, correlated with vigorous growth.

"One seems justified in concluding that the mycorrhizal fungi, both ectophytic and endophytic, are potential parasites controlled by reactions of the host-cells" (Burges, 1936). Lacking sufficient ionizable substance, the tissue is parasitized and progressively destroyed. Possessing requisite ions the tissue breaks down the fungus.



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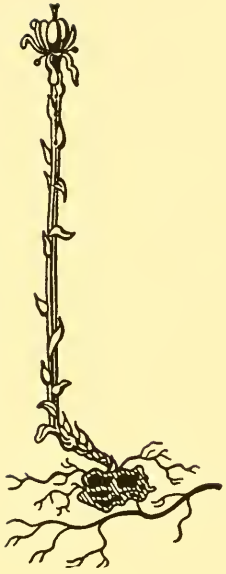
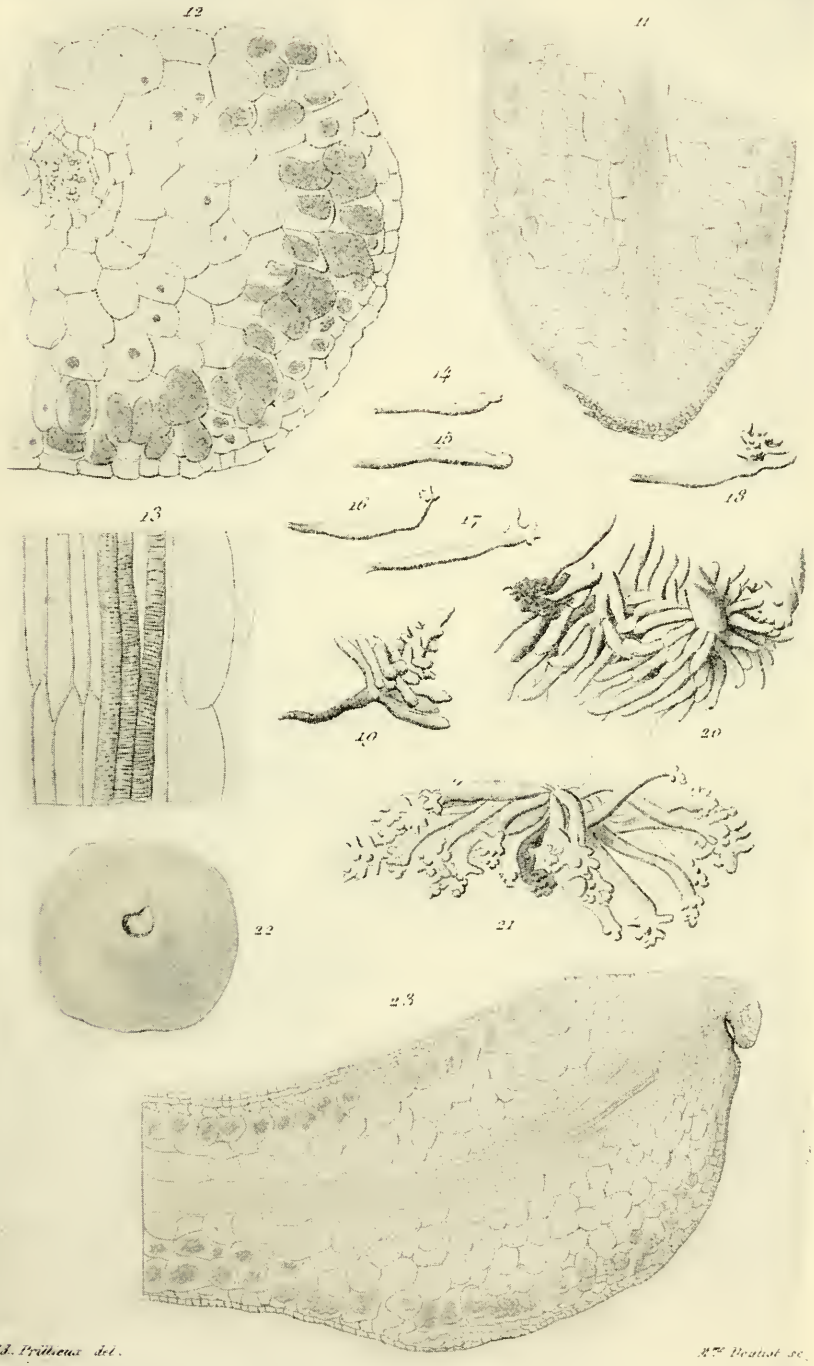


PLATES

PLATE 1.—This plate, prepared by ÉDOUARD PRILLIEUX and published in 1856, is one of the earliest illustrations of mycotrophic infection. It shows the habit of the orchid, *Neottia,* and its fungal infection in figures 7-10. In fig. 7, the outer cortical cells are shown filled with a mycotrophic content; in fig. 8, a septate mycelium; in fig. 9, a mass of yellowish matter surrounded by hyphae; in fig. 10, young cellular tissue containing pelote and intact nucleus. Further stages, and development of coralloid mycorrhizome, were illustrated in Plate 18. (*Reproduced from* Ann. d. sci. nat.: Bot., 4 me. sér. vol. 5, pl. 17, 1856)



Développement des Racines du Neottia nidus-avis.

PLATE 2.—Mycorrhizae in Scot's Pine, *Pinus sylvestris*.—Dr. FRANK started modern mycorrhizal study with a description of such structures in pine and beech. This illustration shows both coralloid and nodulous forms of mycorrhizae, and was originally published by MELIN. (*Reproduced from* RAYNER'S Mycorrhiza, Plate VI).

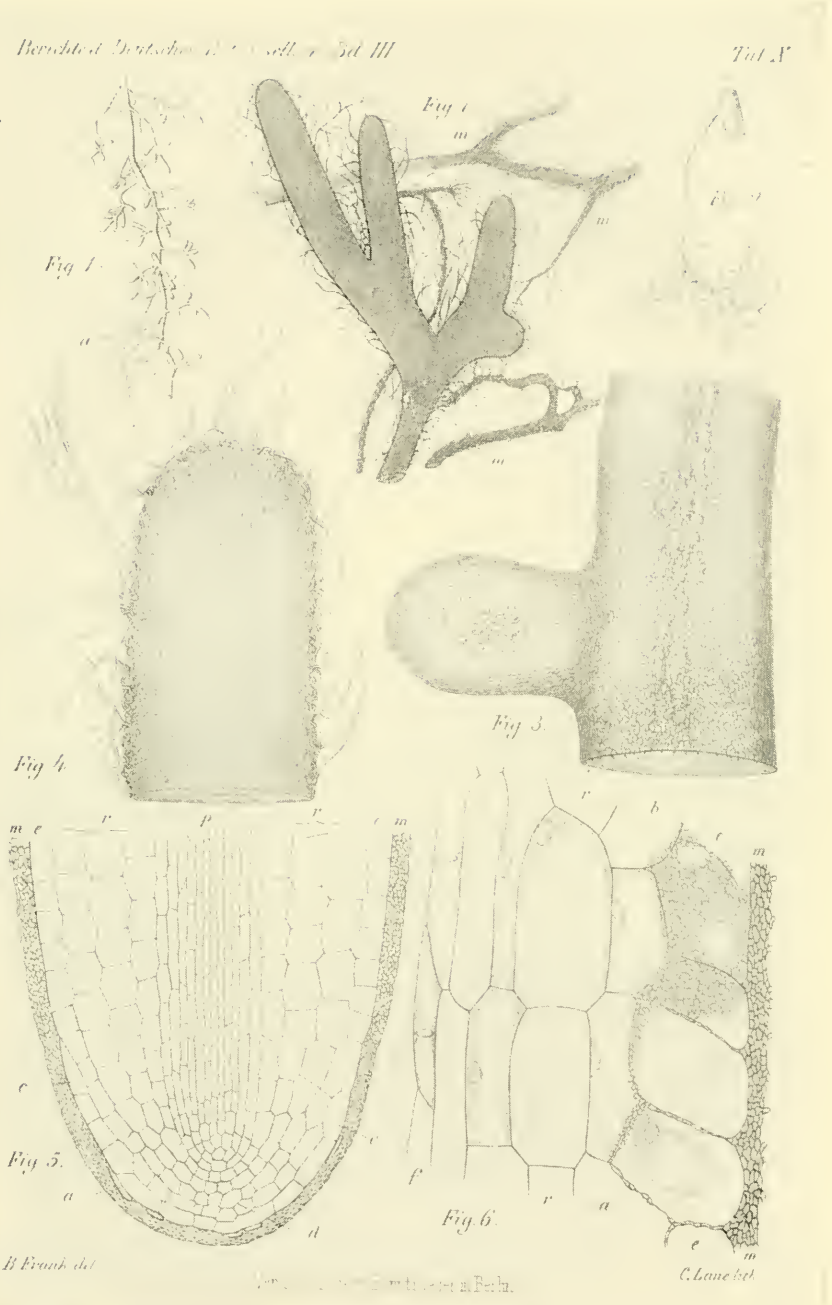
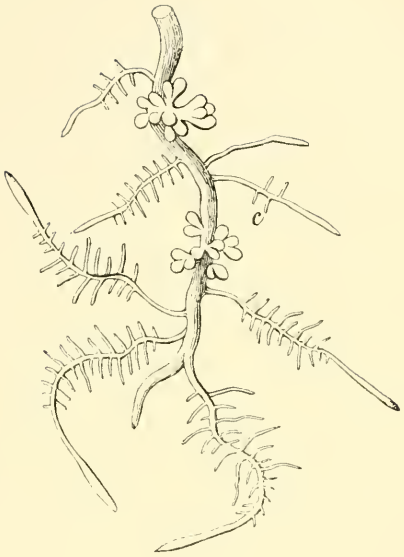


PLATE 3.—The plate which accompanied FRANK'S epochal paper. All of the figures except 4 and 7 are to illustrate mycorrhizae of *Carpinus Betulus*, the other two being of *Fagus sylvatica*. The mycotrophic enlargement of the rootlets, and mantle of hyphae are well shown; and the sections neatly indicate ectotrophic structure. (Ber. deut. bot. Gesell., vol. 3, pl. 10).

PLATE 4.—Effect of mycorrhizae on plant growth. "Two beds of seedlings of a Himalayan species of pine, *Pinus longifolia,* from the same sowing in northern Rhodesia. Further bed inoculated with soil containing a mycorrhiza-forming fungus for the species from a vigorous plantation of this pine at a station in Southern Rhodesia 1000 miles distant; nearer bed untreated." (*From* a photograph kindly loaned by Dr. RAYNER).

PLATE 5.—Photomicrograph of a portion of a fossil mycorrhizome of *Scleropteris illinoiensis*. In the large cortical cell are hyphae and bodies that are considered as vesicles. (*After* ANDREWS and LENZ in Torr. Bull. 70:122. 1943).



INDICES

Subject Index

Acid soil 5,62,63,69,78,83,85
Actinomycosis 113
Adventive roots 57,108,117
Aeration 104
Aerial organs 68,101,102,117,139
Africa 59
Alder 31,54,56,72
Algae 17,24
Alkaline soil 5,69,73,78,83,85
Alpine 57,93
Altitude 80,86,112
Aluminium plants 69,70
Ammonia content of soil 76
Anatomy 84
Aneurin 10,160,180
Annuals 80,82,117,137
Antheridia 23
Apetalae 30
Apex 22,82,92,99,106,110,119,120,170,176
Arbuscle 3,9,85,93,109,125,129,167,179
Archegonia 23,92,93,176
Arctic 57
Aridity 74,88
Arthrophytes 14,21
Ascomycete 40,97
Asia 60
Asparagin 76
Aspen 74
Asymbiosis 104,108,110
Asymbiotic germination 7,142,143
Australia 63
Austria 58
Autotrophic 39,153
Auxone 10

Bacteria 23,24,25,30,70,103,104,105,106,107, 108,109
Baltic States 56
Basidiomycete 38,116,142
Beech 31,40,55,56,57,69,71,73,76,81,83,121,148
Björkmann's hypothesis 157
Bohemia 58
Brazil 64
British Isles 55
Bryophytes 18
Bulb 104

Cacti 74
Calcareous soil 69
Calcium salts 154
Canada 65
Carbohydrate 144,152,158,178
Carbon dioxide 79,155,158
Carbon hypothesis 10,146,158,159,178
Carbon supply to embryo 145
Carbonic acid 10
Caryopsis 8,63,101
Central U.S.A. 66
Ceylon 60
Chaparral 59,67
Chile 64
Chlorophyll tissue 92,93,176
Chlorosis 28
Clay 67,69,74,93
Climatic influence 7
Clot 3,18,93,98,100,127,165
Coal-ball 47
Cold, effect of 82
Colours of mycorrhizae 118
Communication hyphae 119,150,156,163
Composts 89
Conifer 23,25,56,62,63,76,81,88,149,171,177
Consortium 23,30
Coralloid 24,41,69,72,99,108,109,116,117,127, 131
Corm 104
Cortex 20,23,29,49,50,51,75,82,83,95,99,101, 104,105,106,108,109,110,123,130,132,157, 167,174
Croatia 58
Cryptogams 40,61
Culture experiment 139,149,160

Degeneration 7,142
Denmark 55
Diarch bundle 51
Dichotomy 6
Dicotyledons 33,61,125
Digestion: See Phagocytosis
Digestion cells 98,99,115,151,157,170,172
Digestion stages 8,93,95,97
Disease 150,152,164
Distribution 53,75

Ecology 5,12,24,29,137
Ectendotrophic 123,172
Ectotrophic 29,30,59,61,62,64,67,70,71,75,78, 80,84,108,122,123,155,171
Edaphic 70
Eiweisshyphen 98,126,157
Endophyte 4,7,12,18,23,29,37,39,41,44,54,60, 66,80,91,93,97,101,102,103,111,112,115, 116,123,140,145,149,157
Endophytism 46

Endotrophic 21,23,25-28,32,34,40,56,58,59,61-62,70,72,75,80,84,85,87,97,103,105,108,114,116,122,125,149,157,162
Enzyme 25,105,108,109,143,166
Epidermis 50,94,97,109,114,119,123,130,168,169,170
Epiphytic 63,68
Ericads 56,61,62,65,66,72,78,110,116,125,130,138,139,148,149,169
Exkretkörper 109

Facultative mycotrophy 114,115,138,149
Fairy rings 76
Fern prothallia 40,66,146,177
Ferns 4,20,49,52,94,97
Finland 56
Forest fungi 63,136
Forest tree mycorrhizae 7,11,38,55,61,73,87,136,153
Form genera 43,45
Fossil mycorrhizae 47
France 54
Freezing 79
Fungal identity 8,41
Fungistatic substances 90
Fungus 23,30,48,68,77,99,104,107,109,110

Gametophyte 14,19,22,23,94,175
Gasteromycete 43
Germany 53
Germination 44,56,77,139,141,149
Gliotoxin 90
Glomerule 168
Glycogen 100
Grasses 64
Growth-promoting substances 89,100,160
Guatemala 165
Gymnosperm 14,23,60,61

Habitat 39,54,84,132
Halophyte 58
Hartig net 5,26,27,49,119,123,134,133,172
Hatchian hypothesis 9,155
Heather 35
Helotism 152
Heminasidiomycete 41
Hepatic 14,17,22,48,56,58,60,61,157,158,175
Herb 32,38,53,54,57,60,66,73,83,125,137,175
Heteroauxin 10
Historical geology 47
Host-cell 50,98,105,115,152
Humus 2,3,5,12,42,54,67,68,70,71,73,89,92,104,113,115,119,136,144,153
Hungary 58
Hydrocarbon hypothesis 159
Hydrogen ion 3,9,99,152,169,180,182
Hygromorphic 61,69
Hygrophyll 35
Hypertrophy 85,92,99,103,105,109,111,121,124,168
Hyphae 17,20,22,25,49,51,91,94,105,119,123,128

Identity mycorrhizal fungi 8,38,67
Immunity 182
India 60
Inoculation of soil 12,88,89
Insectivorous plants 33
Intaking mechanism 161
Intercalary vesicles 51
Intercellular 22,111,125,128,129,134,167
Intracellular 20,22,50,95,101,106,115,118,123,127,168,170,173
Ions, balancing of 11,181
Isolation 8
Istria 59
Italy 59

Japan 43,62
Java 60

Law optimum altitude 80
Legumes 39,57,60,62,64,103
Lichen symbiosis 17
Light, effect of 80
Limitation endophyte 91,92,94,174,177
Liverwort 3,4,14,17,40,62,91,146,157,158,175
Loam 73,114
Long-root 82,116,176
Lycopod 14,94,95,146
Lycopsida 21,39,50,52,61,177

Madagascar 60
Mamelon 25,26
Mamillae 130
Mantle 29,111,119,121,123,130,169,172
Manure 71,80,90
Maqui 59
Marsh plants 74
Meristem 22,120,121,170,176
Mesic species 175
Microflora 79
Microhabitat 12,84
Mineral intake 9,154,155
Mineral nutrients 9,11,70,77,153,155,160
Monarch bundle 135
Monocotyledons 35,61,125,127
Mother-root 104,116
Mull 70,72
Multiple mycorrhiza 37
Muskeg 48
Mutualism 17,26,51,95,103,148,149,164
Mycocaryopses 8,101
Mycocecidium 103,142
Mycoclena 119,124,125
Mycoderm 119
Mycodomatia 30,103,142
Mycophagy 151
Mycorrhiza, definition 6
Mycorrhizae, early observers 5
Mycorrhizal form 39,71,74,116,117
Mycorrhizome 4,23,49,50,59,64,91,97,99,100
Mycothalli 4,17,21,22,58,91,132
Mykokrinie 70

Netherlands 55
New Zealand 42,62
Nicotinic acid 160
N assimilation 24,61,87,104
N atmospheric 78
N fixation 25,69,78,104,105,106,108,109,110,132
N nutrition 7,9,61,153
N salts 7,9,45,71,76,77,86,153

N theory 9,153
Nodules 2,24,25,26,31,41,62,103-105,108-111, 114,132
Northeastern U.S.A. 65
Norway 56
Nucleic acid 76
Nucleus 22
Nursery 86-88
Nutrient conveyors 163
Nutrient salts 15,68,154
Nutrition, mycorrhizal 98,137,151

Obligate mycotrophy 8,91,110,136,138,140
Occurrence 14,16
Orchid 3,7,36,39,58,60,62,64-66,77,79,83,97, 104,115,125,140,149,157,158,160,164,174
Orchid fungi 7,141,142
Orchid seed germination 44,141
Oxidase level 180

Pacific coast 67
Palaeobotany 47
Palmette 124,134
Parasitism 2,5,6,9,21,23,38,50,51,71,74,84,105, 107,112,118,149,156
Passage cell 132,169
Pathogen 149
Pearl-necklace mycorrhiza 25-27,68,69,76,117
Peat 67,90,95
Pecan mycorrhizae 66,73,74,83,119
Peloton 20,39,92,96,114,115,126,165
Peptone 76
Perennism 7,8,80
Peritrophic 84,85
Phagocytosis 3,8,11,26,50,51,93,99,100,105,107, 110,111,127,129,143,164,170,177,180
Phallomycete 43
Phenology 81-83,114,122,171
pH reactions 77,85,143,166
Phycomycete 11,33-35,38,39,47,54,57,116,119
Phytophagy 7,170
Pilzwirthzellen 50,98,126
Pinaceae 26
Pine 23,26,29,38,42,45,54,55,57,63-65,67,69,71, 74,77,82,83,86,89,121,131,154,174
Pioneer-root 116
Plasmoptyse 130,169
Phosphoric complexes 179
Podsol 73
Portugal 55
Potassium salts 45,77,87,154,158
Potato 37,44,59,80,104,111,150
Prairie 12,86-88
Prosporidi 25,148,174
Protein hyphae 98,100,167,172
Proteolysis 45,164,182
Prothallia 20,21,40,48,63,91,146,175
Pteridophyte 14,19
Pteridosperm 50
Pseudomycorrhizae 66,69,74,118,164
Pure culture 28

Racemose 24,69,117
Rain 75,76,83,121
Raw humus 70,72
Renewed growth 69,74,76,82,83,116,121
Rhizoids 22,91,92,95,97
Rhizomorph 71,119
Rhizosphere 12,84,85
Rhizothamnion 72,117,132
Rocky Mountains 67
Romellian hypothesis 152
Root-cap 20,120,121
Root competition 70
Root-hair 1,5,12,15,20,24,25,27,28,50,53,98, 109,119,150,156,162
Rosette formation 107

Salt absorption 155
Salt marsh 51,58,85
San Domingo 65
Sand 12,56,67,69,71-75,93,105,108,114
Sap concentration 112,165
Saprophyte 5,38,50,52,74,84,101,104,140
Seed coat 101
Seed infection 8,139
Seed sterilisation 139,143
Seedlings 76,79,80,85,86,88-90,107,139,142,154. 155
Seeds, impotence 145
Sekretkörper 107
Setose hyphae 117,119,121,162
Short-root 116,118
Sicily 59
Simple mycorrhiza 117
Slope exposure 75
Soil 12,68,69,72,73,74,76-78
Soil inoculation 28,60,64,88,89
Solfatara 62,69,75
South America 64
Southern U.S.A. 66
Sporangiole 20,91,93,105,125,127,129,167
Spore colour 43
Sporophore 4,8,38,67,76
Sporophyte 19,22
Spruce 23,29,56
Stahlian hypothesis 19,155
Staining 62
Starch 3,17,91,95,99,100,108,110,114,130,151, 156,167,177
Starch, hydrolysis 143,178
Stele 48,49,51,100,109,111,114,120,134,174,177
Sugar 7,71,78,108,110,112,113,139,143,144,156, 169,178
Sugar-cane 65
Symbiophile 38
Symbiosis 109,136,164
Synthesis 9,29,31,38,40-43,107,131,136,142,149
Synthetic mycorrhizae 128
Systemic infection 44,140

Tannin 108,111,172,174,175
Taxonomic identity 21
Thamniscophagous 85
Theories of mycotrophy 9,148
Thiamin 160,166
Toxic residues 72,90
Toxin 115,139,149,164,180
Transpiration stream 9,70,154,156
Tree mycorrhizae 77,83,90,136
Trenching experiments 29,90
Trinidad 65
Tropical 60,65
Truffles 40

Tuber 93,103,104,108,111,113,115
Tuber formation 7.79,80,115,146
Tuber, microscopic 113
Tubercle 23,24,51,104,106,107,111
Tuberous mycorrhizae 93,109,113,117,131

Vascular structure 51
Venezuela 65
Verdauungszellen 50,99,126
Vesicle 39,48-51,85,92-94,97,99,109,123,128, 130,169
Vesicular-arbuscular mycorrhiza 11,47,48,103, 126,127,167
Vitamin 10

Wall tubule 114,132,165
Water intake 70
West Indies 65

Xeric species 75
Xeromorphy 61.69

Index of Plant Names

Abies 28,121,123,131,133
Abietineae 125
Abelicea 31
Acer 25,34,60,117,150
Acorus 35
Actinomyces 106,107
Adenostoma 67
Adenostyles 54,113,134
Adiantum 21,66,97
Aesculus 34,120
Agaricaceae 41,137
Agaricus 41
Agave 35
Ailanthus 33,34,109
Albizzia 61
Aletris 36,62
Alisma 35
Allium 35,56,115,164,170
Alnus 31,56,62,72,105,107,108,109,118,121,123, 136
Aloë 35
Alsophila 20
Alternaria 138
Altingia 34
Amanita 37,41,42,56,78
Amanitopsis 42
Amaranthus 53
Amaryllidaceae 36
Amentaceae 137
Amyelon 51
Amygdalus 11,34,69,177
Anacardiaceae 34
Ananas 36
Andreales 19
Andromeda 111,130
Aneura 4,94
Angiopteris 19,125,136
Anonaceae 34
Anthoceros 18
Aplectrum 67,104
Apocynaceae 169
Apteria 65
Arachnites 57
Araliaceae 34
Araucaria 28,89,125
Arbutus 59,111,130,134
Archangiopteris 20
Arctostaphylos 67,125
Ardisia 34
Arenaria 32
Arisaema 35
Armeria 85
Armillaria 42,100
Aronychia 174
Arum 10,35,40,125,160
Asarum 32
Asclepiadaceae 58,169
Asclepias 34,58,132
Asimina 34
Asparagus 35,114
Aspergillus 40,78
Aspidium 21
Asplenium 21
Aster 68
Asterocystis 40
Asteroxylon 48
Atriplex 32
Azalea 139

Bacillus radicicola 24,104,109
Begonia 168
Benzoin 33,34
Beta vulgaris 32
Betula 56,62,73,134
B. alba 55,63,136,172
Bignonaceae 33
Blechnum 21
Boehmeria 32
Boletus 28,37,38,41,56,57,58,60,71,78,87,89,136. 137

Borraginaceae 33
Botrychium 19,39,66,94,95,97,129,132,175,177
Bromeliaceae 36
Burmannia 5,36,61,65
Buxus 34

Cacao 35,65,128,176
Cactaceae 34
Caesalpinaceae 34
Calla 35
Calluna 8,35,44,55,69,73,78,100,102,111,130, 134,138,139,149,153,170
Calycanthaceae 33,34
Calypogeia 92,93
Calypso 19,99
Calvatia 43
Campanula 33
Cantharellus 42
Caprifoliaceae 34
Carex 35
Caragana 89
Carpinus 31,55
Caryophyllaceae 32
Castanea 54,55,123,136,176
Castanopsis 31
Casuarina 60,61,63,66,89,105,109
Cattleya 44,142,143
Ceanothus 66,109
Cedrus 26,58,59
Celastrus 34,67
Centrospermae 30
Celtis 25,31,49
Centrosis 59,174,177
Cephalanthera 58,100
Cephalotaxus 26
Cerastium 32
Ceratopteris 19
Cercocarpus 67
Chamaecyparis 27
Cheiropleuria 21,62,95,146
Chenopodium 32
Cinchona 61
Cistaceae 33
Citrus 34,40,60,67,83
Clathrus 43
Clethra 34
Clitocybe 42
Cochlearia 127
Compositae 33
Comptonia 30
Conocephalus 18,93,128,158,175,176
Convallaria 35,83
Corallorhiza 55,58,67,99,100,127,162,176,177
Cordaites 50
Coriaria 62,110
Cornaceae 34
Corticium 44
Cortinarius 41,42,43
Corylus 31,42
Cotylanthera 61,177
Cruciferae 33
Cryptogams 61
Cryptomeria 68
Cunninghamia 28
Cupania 34
Cupressus 27
Cuscuta 145
Cyathea 20
Cycadaceae 24
Cycas 104
Cydonia 34
Cymbidium 143,145
Cyperaceae 35
Cyperus 35,114

Dahlia 113
Danaea 20
Daphne 34,58
Daucus 110
Dendrobium 174
Dianthus 32
Diapensia 33,34
Dicotyledons 61
Dioscorea 35
Diospyros 34
Dipodium 63,132,176,177
Drosera 33,62
Dryas 57,80,124,137
Dysoxylon 34,167,174

Eichhornia 120
Elaeagnaceae 34
Elaeagnus 109
Elaeocarpus 174
Elaphomyces 40,41,54,75,136
Empetrum 78
Encephalartos 24
Endogyne 39,40
Entorrhiza 41,114
Ephedra 30
Epigaea 35,65,102
Epilobium 33
Epipactis 58,143
Epipogon 127
Epirrhizanthes 61
Erica 130,149
Ericaceae 34,35,44,57,72
Eriophorum 35,114
Eriostemon 63
Erythronium 35
Eucalyptus 34,43,60,63,64,110,173,174
Euphorbia 34,49

Fagaceae 31
Fagus 55,69,71,73,118
Fegatella 18,92
Ficus 32
Firmiana 34
Forsythia 59
Fossombronia 93,94,146
Fragaria 44,54,175
Frankia 107,109
Fraxinus 35,84,85,117,173
Fritillaria 35
Fusarium 43,44,45,111,115

Galanthus 35
Galeola 62,79
Gardenia 34
Garryaceae 30
Gastrodia 62,63,100,137,140,141,153,158,177
Geaster 43
Gentiana 34,35,80,132
Ginkgo 24,26

Glaux 85
Gleicheniaceae 20,95
Glyceria 85,161
Gonyanthes 174
Goodyera 144,145,175
Gossypium 35,128
Gramineae 35,39
Gymnosperms 61
Gypsophila 32
Gyrodon 107

Habenaria 141
Hamamelis 34
Haplomitrium 56
Hebeloma 3,56
Helicia 168
Helleborine 141
Helminthostachys 19,95
Hemerocallis 35
Hemibasidiomycetes 41
Hevea 34
Hicoria 30,66,73,83
Hippophaë 56,84,110,176
Hoja 132
Humaria 40
Hydnum 42
Hygrophorus 42,137
Hymenophyllaceae 19
Hypericum 33
Hypholoma 29
Hypomyces 44

Ilex 34
Iridaceae 36

Juglans 30,49,117
Juncaceae 145
Juncus 35,41,85,113,114,135
Jungermanniaceae 17,18,93,157
Juniperis 26,56

Kaulfussia 20

Labiatae 33
Lactarius 38,41,42,78,136,137
Laelia 143
Larix 29,102,123,134,136,172
Lathraea 107
Lauraceae 34
Lecanorchis 174,177
Ledum 130
Leguminosae 34,160
Leiophyllum 66
Leitneria 30
Lepidodendron 48
Lepiota 42
Leucobryum 40
Leucojum 35
Librocedrus 28
Liliales 35,39
Linodorum 141
Linum 33
Liquidambar 25,34
Liriodendron 34,117
Litchi 60
Lithocarpus 31
Livistona 35
Lobelia 64,152
Lolium 8,10,35,55,63,64,67,101,102,128
Lotus 54
Lunularia 18,45,60,78,92,93,146,175,176
Lycoperdon 43
Lycopodium 22,61,65,95,96,97,175,176

Macroglossum 95
Macrozamia 24,63
Magnolia 34
Maianthemum 35,83
Malaceae 34
Mangiletia 34
Marasmius 42
Marattiaceae 4,19,20,95
Marchantia 18,92,93,146
Marchantiaceae 17,18,93,157,175
Marsilea 19
Matoniaceae 19
Melampyrum 113
Melanthaceae 35
Melia 34
Menispermaceae 133
Merati 34
Michelia 34,174
Mimosaceae 34,108
Molinia 40,56,114
Mollisia 40
Monoclea 62,92,129,175
Monocotyledons 61
Monotropa 2,3,6,9,34,78,98,121,130,138,149, 151,169,170,176
Mortierella 78
Morus 31
Musa 35,36
Muscari 35
Mycelium radicis 7,45,141
M.r. abietis 45
M.r. atrovirens 45,160
M.r. Fagi A 78
M.r. nigrostrigosum 45,118
M.r. sylvestris 45
Myosurus 54
Myrica 30,61,62,65,106,107
Myrsinaceae 33

Narcissus 35
Nectria 44
Negundo 34,126
Neottia 2,3,6,9,34,98,99,126,141,150,151,165, 175,176
Nephrolepis 21
Neomammillaria 34,123
Nerium 34
Nothofagus 42,63
Nyssa 33,34

Oboloria 34,75
Ocotea 34
Odontoglossum 44,143,144
Olea 174
Oleaceae 34
Omphalia 42
Onoclea 21
Oospora 19,44
Ophioderma 174
Ophioglossaceae 4,19,56
Ophioglossum 19,44,61,95,125,129,177

Ophyrs 143
Orchcomyces 7,45,142
Orchidaceae 56
Orchis 44,75,84,100,141,145,165,177
Ornithogalum 35
Orobanche 113,145
Orobus 109,127
Oryza 74
Osmundaceae 20,95
Osmundites 49
Ostrya 31
Oxalis 33
Oxycoccus 35,78

Palaeomyces 48
Pandanus 35
Papilionaceae 33
Paris 125,176
Parkeriaceae 19
Paxillus 78
Pellia 17,18,45,55,78,91,93,146,175,176,177
Penicillium 40,78
Peronosporites 48,51
Petallophyllum 94
Phalenopsis 143,145
Phascum 19
Pherosphaera 26,63
Philesia 64,176
Phoenix 35
Phoma 7,44,45,78,140
Phyllophoma 44,138
Phyllostachys 43
Phytolacca 32
Picea 29,43,65,78,102,131,134,137,172
Picrasma 34
Pilularia 19
Pinaceae 125
Pinus 42,43,57,62,64,66,67,77,82,89,90,120,123, 132,136,154,174
P. densiflora 42,73,121
P. Merkusii 60,89,136
P. montana 29,78,117,131,132
P. pinaster 29,64,136
P. radiata 63,64,77,83,89
P. Strobus 29,43,77,82,86,135
P. sylvestris 29,55,73,78,131,132,134,138,144, 172
P. Taeda 63,75,138
P. virginiana 27,68,75,122
Pithecolobium 108
Pittosporum 33,34
Plantago 58,85
Platanus 34
Platycarpa 30
Plumbaginaceae 33
Podocarpineae 134
Podocarpus 24,25,28,62,63,73,105,125
Podophyllum 33
Pogonia 175,177
Polygala 33,137
Polygonaceae 62
Polygonatum 35
Polygonum viviparum 32,57,108,125
Polypodiaceae 20,21
Polyporus 42
Polysaccum 43
Populus 30,41,57,136,137,172
Portulaca 32
Potentilla 35
Preissia 17
Primula 33
Proteaceae 168
Protomycitis 48
Prunus 34
Pseudolarix 29
Pseudomonas 109
Pseudotsuga 29,81
Psilotaceae 22
Psilotum 23,104,125
Pteridium 21,62,97,171
Pterocarya 30
Pterospora 98,121
Pyrolaceae 56,111
Pythium 21,39,60,61,95

Quercus 31,55,66,67,86,118,123,124,136

Ranunculaceae 33,39
Ranunculus 53
Raphanus 108
Rhamnaceae 34,177
Rhizanthella 64,100,175
Rhizobium 104
Rhizoctonia 7,34,44,45,111,115,137,140,141, 143,164
Rhizonium 50
Rhizophagus 11,40,45,48,63
Rhizopogon 27,78,136
Rhododendron 35,66,139,149
Riccia 18
Robinia 43
Rosaceae 34
Rubus 65

Saccharum 175
Sagina 32
Salicornia 32,58,85
Salix 30,57,62,124,136,150
Salsola 32
Salviniaceae 19
Sapindaceae 34
Sarcodes 67,98,121
Sarcosiphon 63
Sarraceniaceae 33
Sassafras 34,66
Schimzia 107,114
Schizaeaceae 19
Sciadopitys 28
Sciaphila 64
Scilla 35
Scitaminaceae 36
Schoenus 114
Scleranthus 32
Scleroderma 29,43
Scrophulariaceae 33
Selaginella 22
Sempervivum 121
Sequoia 28,125
Sewardiella 92,93,145,176
Shepherdia 109
Silene 32
Smilacina 35
Smilax 35
Solanum 35,64,112

S. tuberosum 80,111,112,113
Sphagnum 19
Spiranthes 83,99,143,145
Stapelia 128
Staphyleaceae 34
Stellaria 32
Sterculiaceae 33,34
Stigmaria 48
Streptothrix 19
Strobilomyces 42
Styrax 34
Suaeda 58,85
Symplocos 34

Talauma 34
Tamaricaceae 33,34
Tamus 104,125
Taxodium 28
Taxus 26,55,69,82,122,152,174,176
Terfezia 40
Thea 34,35
Thismia 66,87,98,100,176
Thuja 28,58
Thuopsis 28
Tilia 34,82,123
Tilletiaceae 41,114
Tipularia 66,104,132
Tmesipteris 4,23,39,97,134
Todea 20
Torreya 24,26
Trametes 54
Tribulus 56,108,151,177
Tricholoma 37,41,42
Trigolochin 85
Trillium 35
Tuber 40,41,147
Tulipa 35
Typha 35

Ulmus 31,32
Urtica 32
Uvularia 35

Vaccinium 34,35,110,130,139
Vanda 160
Vanilla 68
Veratrum 35
Vinca 128,132,152,168,176
Viola 33,39,56
Vitaceae 34
Vitis 83,84,128,168,176

Yucca 35

Zelkova 31
Zeuxine 66,137
Zingiber 36
Zöopsis 92

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